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08/983605

Atty's Docket No.  
2936.104/00

21 Rec'd PCT 29 DEC 1997

## EXPRESS MAIL CERTIFICATION

"Express" Mail label number: **M58 48430 24**

(A) Date of Deposit: December 29, 1997

I hereby certify that this transmittal letter and the papers and fees identified in this transmittal letter as being transmitted herewith are being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. 1.10 on the date indicated at (A) above and are addressed to the Assistant Commissioner of Patents, Washington, D.C. 20231

Name of Person mailing the above: Kathleen D. Monical

Signature of Person mailing the above item

TRANSMITTAL LETTER TO THE UNITED STATES  
DESIGNATED/ELECTED OFFICE (DO/EO/US)

International Application No.: PCT/DE96/01185  
International Filing Date : 27 June 1996 (27.06.96)  
Priority Date Claimed : 28 June 1995 (28.06.95)  
Title of Invention : Microsatellite Markers for  
Plants of the Species Triticum  
Aestivum and Tribe Triticeae and  
the Use of Said Markers

Applicant(s) for DO/EO/US : Marion Roder; Jens Plaschke;  
and Martin Ganal

Applicant herewith submits to the United States Designed/Elected Office (DO/EO/US) the following items under 35 U.S.C. 371:

1. ☒ This express request to immediately begin national examination procedures (35 U.S.C. 371(f)).
2. ☒ The U.S. National Fee (35 U.S.C. 371(c)(1) and other fees as follows:

|  |                         |                             |   |
|--|-------------------------|-----------------------------|---|
| TOTAL CLAIMS<br>10 - 20 =  | CLAIMS<br>OVER 20<br>-- | RATE<br>X \$22 =            | TOTAL FEES FOR<br>CLAIMS OVER 20<br>--            |
| NUMBER OF<br>INDEPENDENT CLAIMS<br>1 - 3 =   | CLAIMS<br>OVER 3<br>--  | RATE<br>X \$80 =            | TOTAL FEES FOR<br>INDEPENDENT CLAIMS OVER 3<br>-- |
| MULTIPLE DEPENDENT<br>CLAIM(S) PRESENT<br>No   |                         | RATE<br>\$250 per<br>APPLN. | FEE<br>MULTIPLE DEPENDENT CLAIM(S)<br>\$ --       |
| BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(4)):<br>— International preliminary examination fee paid to<br>USPTO (37 CFR 1.482) = \$ 700.00<br>— No International preliminary Examination fee paid to<br>USPTO (37 CFR 1.482) but international search fee<br>paid to USPTO (37 CFR 1.445 (a)(2)) = \$ 770.00<br>— Neither International preliminary examination fee<br>(37 CFR 1.482) nor International search fee<br>(37 CFR 1.445(a)(2)) paid to USPTO = \$1040.00<br>— International preliminary examination fee paid to<br>USPTO (37 CFR 1.482) and all claims satisfied<br>provisions of PCT Article 33(2)(2) to (4) = \$ 96.00<br>x Filing with an EPO or JPO search report = \$ 930.00 |                         |                             | \$ 930.00   |
| Surcharge of \$130.00 for furnishing the national fee<br>or oath or declaration 20 mos. from the earliest<br>claimed priority date (37 CFR 1.482(e)).  |                         |                             |   |
| TOTAL OF ABOVE CALCULATIONS  |                         |                             | \$ 930.00   |
| Reduction by 1/2 for filing by small entity  |                         |                             |   |
| SUBTOTAL   |                         |                             | \$930.00  |
| Process fee of \$130.00 for furnishing the English<br>translation later than 20 mos. from the earliest<br>claimed priority date (37 CFR 1.482(f))  |                         |                             |   |
| TOTAL NATIONAL FEE   |                         |                             | \$ 930.00   |
| Fee for recording the enclosed assignment  |                         |                             |   |
| TOTAL FEES ENCLOSED  |                         |                             | \$ 930.00   |

- a. ☒ A check in the amount of \$930.00 to cover the above fees is enclosed.
- b. ☐ Please charge my Deposit Account No. 04-2216 in the amount of \$ to cover the above fees. A duplicate copy of this sheet is enclosed.
- c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 04-2216. A duplicate copy of this sheet is enclosed.

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3. A copy of the International Application as filed (35 U.S.C. 371(c)(2))
  - a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
  - b. ☐ is not required, as the application was filed in the United States Receiving Office.
  - c. ☒ has been transmitted by the International Bureau.
4. ☒ A translation of the International Application into English.
5. Amendments to the claims of the International Application under PCT Article 19
  - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
  - b. ☐ have been transmitted by the International Bureau.
6. ☐ A translation of the amendments to the claims under PCT Article 19
7. ☐ An oath or declaration of the inventor [35 U.S.C. 371(c)(4)]
8. ☐ A translation of the Annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Other document(s) or information included:

9. ☒ A Preliminary Amendment
10. ☐ An assignment document for recording. Please mail the recorded assignment document to the undersigned.
11. ☐ The above checked items are being transmitted
  - a. ☐ before the 18th month publication.
  - b. ☐ after publication and the Article 20 communication but before 20 months from the priority date.
  - c. ☐ after 20 months (surcharge and/or processing fee included).  
 Note: Petition to revive (37 CFR 1.137(a) or (b)) is necessary if 35 U.S.C. 371 requirements submitted after 20 months and no proper demand for International Preliminary Examination was made by 19 months from the earliest claimed priority date.
  - e. ☒ by 30 months and a proper demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
  - f. ☐ after 30 months (surcharge and/or processing fee included).  
 Note: Petition to revive (37 CFR 1.137(a) or (b)) is necessary if 35 U.S.C. 371 requirements submitted after 32 months and a proper demand for International Preliminary Examination was made by 19 months from the earliest claimed priority date.
12. At the time of transmittal, the time limit for amending claims under Article 19
  - a. ☐ has expired and no amendments were made.
  - b. ☐ has not yet expired.
13. ☐ Certain requirements under 35 U.S.C. 371 were previously submitted by the applicant on \_\_\_\_\_ namely:

Please direct all communications in connection with this application to the undersigned at

LONDA AND TRAUB LLP  
20 Exchange Place, 37th Floor  
New York, N.Y. 10005

  
\_\_\_\_\_  
Bruce S. Londa (33,531)

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RECEIVED 29 DEC 1997

M 58 484302#  
08/983605

PATENTS  
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Atty's Docket No: 2936.104/00

Applicant(s) : Marion Roder  
Filed : Concurrently herewith  
For : Microsatellite Markers for Plants of the Species  
Triticum Aestivum and Tribe Triticeae and the Use  
of Said Markers

PRELIMINARY AMENDMENT

Hon. Assistant Commissioner of Patents  
Washington, D.C. 20231

Dear Sir:

Prior to examination, please amend the application as  
follows:

IN THE SPECIFICATION

Page 1, between lines 3 and 4, please insert  
--Background of the Invention--;

Page 2, before line 1, please insert  
--Summary of the Invention--;

Page 3, between lines 10 and 11, please insert  
--Detailed Description of the Invention--.

IN THE CLAIMS

Claim 2, line 1, please delete "characterized in that" and  
insert --wherein--;

08/983605 "104" 584

Claim 3, line 1, please delete "characterized in that" and insert --wherein--;

Claim 4, line 1, please delete "characterized in that" and insert --wherein--;

Claim 5, line 1, please delete "characterized in that" and insert --wherein--;

Claim 6 (amended) A method for the preparation of a microsatellite marker of [claims 1 to 5] claim 1 for plants of the Triticum aestivum species as well of the Tribe Triticeae, [characterized in that] wherein hypervariable genome sections (so-called microsatellites), with the help of the polymerase chain reaction (PCR), are amplified, subsequently separated and detected to polymorphous fragments in the presence of two specific primers, which flank a microsatellite sequence to the left and right of each microsatellite locus.

Claim 7, line 1, please delete "characterized in that" and insert --wherein--;

Claim 8, line 1, please delete "characterized in that" and  
insert --wherein--;

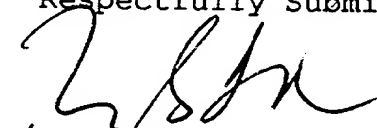
Please cancel claims 9 and 10.

REMARKS

The above amendments were made to place the application into  
proper United States patent format.

Early and favorable consideration of the application is  
respectfully requested.

Respectfully Submitted,



Bruce S. Londa ( 33,531)  
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klm0240

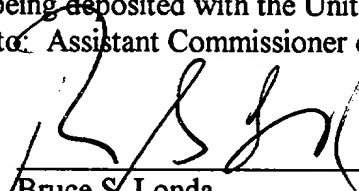
40 Rec'd PCT/PTO 17 FEB 1998



PATENTS

MAIL CERTIFICATION

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Assistant Commissioner of Patents, Washington, D.C. 20231 on February 13, 1998.

  
Bruce S. Londa

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Atty's Docket No.  
2936.104/00

EXAMINER :

GROUP ART UNIT :

APPLICANT : Marion Roder

APPLN. NUMBER : 08/983,605

FILED : December 29, 1997

FOR : Microsatellite Markers for Plants of the Species Triticum Aestivum and  
Tribe Aestivum and Tribe Triticeae and the Use of Said Markers

SUPPLEMENTARY PRELIMINARY AMENDMENT

Hon. Assistant Commissioner of Patents  
Washington, D.C. 20231

Sir:

Prior to examination, please amend the application as follows:

**IN THE SPECIFICATION**

Page 13, line 7, please delete "mappings" and insert --mapping--; and delete  
"distinguishing" and insert --trait analysis--;

08983605-050150

line 3, delete "features";

line 10, please delete "this" and insert --these--; and delete "marker" and insert --markers--;

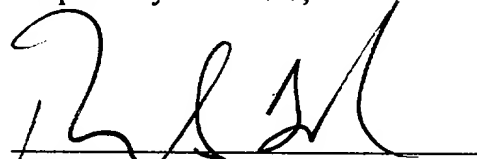
Page 14, line 3, please delete "minute" and insert --minutes--;

Page 15, line 24, please delete "minuutes" and insert --minutes--.

#### REMARKS

The above amendments were made to correct grammatical and translation errors. Early and favorable consideration of this application is earnestly solicited.

Respectfully Submitted,



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2936.104/00

**MICROSATELLITE MARKERS FOR PLANTS OF THE SPECIES**  
**TRITICUM AESTIVUM AND TRIBE TRITICEAE**  
**AND THE USE OF SAID MARKERS**

The invention relates to novel genetic markers for wheats (*Triticum aestivum* L.) and closely related species (Tribus Triticeae) and to the use of said markers.

The most widely spread, known, DNA-based genetic markers are the so-called restriction fragment length polymorphisms (RFLP) markers. For using these markers, genomic DNA is digested with restriction enzymes, separated on agarose gels and transferred to nylon membranes (Southern Blot). Specific fragments are detected by hybridization with radioactively labeled DNA probes. When mutations occur in the region of the restriction enzymes used or when smaller deletions/insertions occur, polymorphisms between different lines are found, which are passed on stably and mostly codominantly. The use of RFLP markers in hexaploid cultivated wheat is possible only to a limited extent, since only very little polymorphism is detected in wheat in this manner.

It has already been described that microsatellite markers detect significantly more polymorphism between different wheat lines than do RFLP markers. This can be attributed particularly to the occurrence of multiple alleles per locus (Röder et al., Mol. Gen. Genet. (1995) 246, 327 - 333). Moreover, it is known that microsatellite markers have the advantage that they can be detected by way of PCR and that therefore large amounts of samples can be analyzed more easily.



It is an object of the invention to provide novel microsatellite markers for the genetic analysis of plants of the *Triticum aestivum* species, which markers are distinguished by a degree of DNA polymorphism, which is higher than that of other molecular probes, that have been developed previously for the wheat genome.

This objective is accomplished by claims 1 to 10. The inventive markers are based on the amplification of certain hypervariable genome sections, the so-called microsatellites, with the help of their polymerase chain reaction (PCR). For specific amplification, two primers, in each to the case left and the right in the flanking sequences, are required for each microsatellite locus. On the average, these primers are  $20 \pm 3$  bases long and are defined by their sequences. In principle, a microsatellite marker is a sequence tagged site (STS), which is defined by two specific primers. These primers flank, in each case to the left and the right, a so-called microsatellite sequence. A microsatellite sequence is defined as a tandem repetitive repetition of a di-, tri- or tetranucleotide sequence, for example  $(GA)_n$ , in which  $n \geq 10$ . Composite microsatellite sequences also occur, such as  $(GT)_n(AT)_n$ , as well as imperfect sequences, in which individual bases are mutated, such as  $(GA)_nCA(GA)_n$ . Among various lines and varieties, there is variation in the number of repeats at a certain locus. After amplification of the microsatellites, this leads, by means of the specific primers in the flanking sequences, to PCR products of different length and, with that, to polymorphisms. These polymorphisms are passed on stably and can therefore be used as genetic markers. In some cases, null alleles (no visible fragment) also occur, when there are mutations within the binding site for the primers.

The separation and detection of the PCR products obtained can be carried out with different technical variants. For separating the fragments, highly resolving agarose gels, native polyacrylamide gels or denaturing polyacrylamide gels (= sequencing gels) can be used. Depending on the separation system, fragments are detected using ethidium bromide staining, silver staining or, after labeling the PCR

fragments radioactively, using autoradiography. A further, very effective variation for separation and detection consists of the use of an automatic sequencer with dye- or fluorescence-labeled primers. For this purpose, it is necessary to synthesize a dye- or fluorescence-labeled primer from each microsatellite primer pair. PCR amplification results in a labeled product, which can be detected by the sequencing equipment. At the same time, dye- or fluorescence-labeled size standards are also separated for each sample in the same track. After that, special software enable the absolute size of each fragment, which has been separated, to be calculated and, with that, also permits fragments from different gel runs to be compared. With this method, several hundred samples can be analyzed largely automatically in a day.

Pursuant to the invention, microsatellite markers are made available, which contain the following primer pairs with assigned microsatellite sequences or a number thereof and amplify the loci of all chromosomes of the wheat genome and therefore find use for gene marking.

WO 97/01567

PCT/DE96/01185

| WMS Number<br>WMS-Nummer | WMS Primer Left<br>WMS Primer links | WMS Primer Right<br>WMS Primer rechts | Length (bp)<br>in "cs"<br>Länge<br>(bp) in<br>'CS' | Repeat Type<br>Repeat-Typ | Annealing<br>Temperature<br>Annealing-<br>Temperatur |
|--------------------------|-------------------------------------|---------------------------------------|--|---------------------------|--|
| WMS052                   | 5' CTA TGA GGC GGA GGT TGA AG 3'    | 5' TGC GGT GCT CTT CCA TTT 3'         | 150  | GTimp                     | 60 °C  |
| WMS055                   | 5' GCA TCT GGT ACA CTA GCT GCC 3'   | 5' TCA TGG ATG CAT CAC ATC CT 3       | 127  | CTimp                     | 60 °C  |
| WMS057                   | 5' TCG ATT CTG AAA GGT TCA TCG 3'   | 5' CGA TCA AGT AGT TGA AAG CGC 3'     | 224  | AAAAAimp                  | 60 °C  |
| WMS058                   | 5' TCT GAT CCC GTG AGT GTA ACA 3'   | 5' GAA AAA AAT TGC ATA TGA GCC C 3'   | 118  | CA                        | 60 °C  |
| WMS060                   | 5' TGT CCT ACA CGG ACC ACG T 3'     | 5' GCA TTG ACA GAT GCA CAC G 3'       | 211  | CA                        | 60 °C  |
| WMS063                   | 5' TCG ACC TGA TCG CCC CTA 3'       | 5' CGC CCT GGG TGA TGA ATA GT 3'      | 271  | GAA,CA,TA                 | 60 °C  |
| WMS067                   | 5' ACC ACA CAA ACA AGG TAA GCG 3'   | 5' CAA CCC TCT TAA TTT TGT TGG G 3'   | 85   | CA                        | 60 °C  |
| WMS068                   | 5' AGG CCA GAA TCT GGG AAT G 3'     | 5' CTC CCT AGA TGG GAG AAG GG 3'      | 182  | GA                        | 60 °C  |
| WMS070                   | 5' AGT GGC TGG GAG AGT GTC AT 3'    | 5' GCC CAT TAC CGA GGA CAC 3'         | 194  | GT                        | 60 °C  |
| WMS071                   | 5' GGC AGA GCA GCG AGA CTC 3'       | 5' CAA GTG GAG CAT TAG GTA CAC G 3'   | 128  | GT                        | 60 °C  |
| WMS077                   | 5' ACA AAG GTA AGC AGC ACC TG 3'    | 5' ACC CTC TTG CCC GTG TTG 3'         | 153  | CA,GA                     | 55 °C  |
| WMS082                   | 5' ACG TTA GAA GGT GCA ATG GG 3'    | 5' AGT GGA TGC ACC GAC TTT G 3'       | 152  | GT,GAimp                  | 60 °C  |
| WMS088                   | 5' CAC TAC AAC TAT GCG CTC GC 3'    | 5' TCC ATT GGC TTC TCT CTC AA 3'      | 121  | GT                        | 60 °C  |
| WMS095                   | 5' GAT CAA ACA CAC ACC CCT CC 3'    | 5' AAT GCA AAG TGA AAA ACC CG 3'      | 121  | CA                        | 60 °C  |
| WMS099                   | 5' AAG ATG GAC GTA TGC ATC ACA 3'   | 5' GCC ATA TTT GAT GAC GCA TA 3'      | 119  | CA                        | 60 °C  |
| WMS102                   | 5' TCT CCC ATC CAA CGC CTC 3'       | 5' TGT TGG TGG CTT GAC TAT TG 3'      | 143  | CT                        | 60 °C  |
| WMS106                   | 5' CTG TTC TTG CGT GGC ATT AA 3'    | 5' AAT AAG GAC ACA ATT GGG ATG G 3'   | 139  | GA                        | 60 °C  |
| WMS107                   | 5' ATT AAT ACC TGA GGG AGG TGC 3'   | 5' GGT CTC AGG AGC AAG AAC AC 3'      | 195  | CT                        | 60 °C  |
| WMS108                   | 5' CGA CAA TGG GGT CTT AGC AT 3'    | 5' TGC ACA CTT AAA TTA CAT CCG C 3'   | 132  | GTimp                     | 60 °C  |
| WMS111                   | 5' TCT GTA GGC TCT CTC CGA CTG 3'   | 5' ACC TGA TCA GAT CCC ACT CG 3'      | 205  | CT,GT                     | 55 °C  |
| WMS112                   | 5' CTA AAC ACG ACA GCG GTG G 3'     | 5' GAT ATG TGA GCA GCG GTC AG 3'      | 101  | CTimp                     | 55 °C  |
| WMS113                   | 5' ATT CGA GGT TAG GAG GAA GAG G 3' | 5' GAG GGT CGG CCT ATA AGA CC 3'      | 148  | GT                        | 60 °C  |
| WMS114                   | 5' ACA AAC AGA AAA TCA AAA CCC G 3' | 5' ATC CAT CGC CAT TGG AGT G 3'       | 206<br>(177)                                       | GA                        | 60 °C  |
| WMS118                   | 5' GAT GTT GCC ACT TGA GCA TG 3'    | 5' GAT TAG TCA AAT GGA ACA CCC C 3'   | 110  | CA                        | 60 °C  |
| WMS119                   | 5' TGA CTA ACA TCC TTT GTC ACG C 3' | 5' CAT GTC TCA ACC ACC CAC AG 3'      | 181  | GTimp                     | 55 °C  |

|        |                                     |                                     |     |           |       |
|--------|-------------------------------------|-------------------------------------|-----|-----------|-------|
| WMS120 | 5' GAT CCA CCT TCC TCT CTC TC 3'    | 5' GAT TAT ACT GGT GCC GAA AC 3'    | 139 | CT, CA    | 55 °C |
| WMS121 | 5' TCC TCT ACA AAC AAA CAC AC 3'    | 5' CTC GCA ACT AGA GGT GTA TG 3'    | 143 | CA        | 50 °C |
| WMS122 | 5' GGG TGG GAG AAA GGA GAT G 3'     | 5' AAA CCA TCC TCC ATC CTG G 3'     | 149 | CT, CA    | 60 °C |
| WMS124 | 5' GCC ATG GCT ATC ACC CAG 3'       | 5' ACT GTT CGG TGC AAT TTG AG 3'    | 213 | CT, GTimp | 60 °C |
| WMS126 | 5' CAC ACG CTC CAC CAT GAC 3'       | 5' GTT GAG TTG ATG CGG GAG G 3'     | 196 | CA        | 60 °C |
| WMS128 | 5' AGC ACA TTT TAA CAC AGA TA 3'    | 5' ATC TGT GAA ATT TTG AAA AC 3'    | 176 | CA        | 50 °C |
| WMS129 | 5' TCA GTG GGC AAG CTA CAC AG 3'    | 5' AAA ACT TAG TAG CCG CGT 3'       | 221 | GTimp     | 55 °C |
| WMS130 | 5' AGC TCT GCT TCA CGA GGA AG 3'    | 5' CTC CTC TTT ATA TCG CGT CCC 3'   | 113 | GT        | 60 °C |
| WMS131 | 5' AAT CCC CAC CGA TTC TTC TC 3'    | 5' AGT TCG TGG GTC TCT GAT GG 3'    | 131 | CT        | 60 °C |
| WMS132 | 5' TAC CAA ATC GAA ACA CAT CAG G 3' | 5' CAT ATC AAG GTC TCC TTC CCC 3'   | 119 | GA, GAA   | 60 °C |
| WMS133 | 5' ATC TAA ACA AGA CGG CGG TG 3'    | 5' ATC TGT GAC AAC CGG TGA GA 3'    | 118 | CT        | 60 °C |
| WMS134 | 5' CAT GGA ACT TAG ACA GAA TTG 3'   | 5' CAG TAC TTG GTA CTG AAC AGG 3'   | 111 | CA        | 60 °C |
| WMS135 | 5' TGT CAA CAT CGT TTT GAA AAG G 3' | 5' ACA CTG TCA ACC TGG CAA TG 3'    | 143 | GA        | 55 °C |
| WMS136 | 5' GAC AGC ACC TTG CCC TTT G 3'     | 5' CAT CGG CAA CAT GCT CAT C 3'     | 296 | CT        | 60 °C |
| WMS140 | 5' ATG GAG ATA TTT GGC CTA CAA C 3' | 5' CTT GAC TTC AAG GCG TGA CA 3'    | 251 | CT        | 55 °C |
| WMS144 | 5' TTT GCT GTG GTA CGA AAC ATA C 3' | 5' ACT CAC AAA TGT CTA ATA AAA C 3' | 200 | GT        | 50 °C |
| WMS146 | 5' CCA AAA AAA CTG CCT GCA TG 3'    | 5' CTC TGG CAT TGC TCC TTG G 3'     | 162 | GAimp     | 60 °C |
| WMS148 | 5' GTG AGG CAG CAA GAG AGA AA 3'    | 5' CAA AGC TTG ACT CAG ACC AAA 3'   | 163 | CA        | 60 °C |
| WMS149 | 5' CAT TGT TTT CTG CCT CTA GCC 3'   | 5' CTA GCA TCG AAC CTG AAC AAG 3'   | 161 | GA        | 55 °C |
| WMS153 | 5' GAT CTC GTC ACC CGG AAT TC 3'    | 5' TGG TAG AGA AGG ACG GAG AG 3'    | 188 | GA        | 60 °C |
| WMS154 | 5' TCA CAG AGA GAG AGG GAG GG 3'    | 5' ATG TGT ACA TGT TGC CTG CA 3'    | 102 | GA        | 55 °C |
| WMS155 | 5' CAA TCA TTT CCC CCT CCC 3'       | 5' AAT CAT TGG AAA TCC ATA TGC C 3' | 141 | CT        | 60 °C |
| WMS156 | 5' CCA ACC GTG CTA TTA GTC ATT C 3' | 5' CAA TGC AGG CCC TCC TAA C 3'     | 277 | GT        | 60 °C |
| WMS157 | 5' GTC GTC GCG GTA AGC TTG 3'       | 5' GAG TGA ACA CAC GAG GCT TG 3'    | 106 | CT        | 60 °C |
| WMS159 | 5' GGG CCA ACA CTG GAA CAC 3'       | 5' GCA GAA GCT TGT TGG TAG GC 3'    | 192 | GT        | 60 °C |
| WMS160 | 5' TTC AAT TCA GTC TTG GCT TGG 3'   | 5' CTG CAG GAA AAA AAG TAC ACC C 3' | 184 | GA        | 60 °C |
| WMS161 | 5' GAT CGA GTG ATG GCA GAT GG 3'    | 5' TGT GAA TTA CTT GGA CGT GG 3'    | 154 | CT        | 60 °C |
| WMS162 | 5' AGT GGA TCG ACA AGG CTC TG 3'    | 5' AGA AGA AGC AAA GCC TTC CC 3'    | 208 | CA        | 60 °C |

|        |                                     |                                     |      |       |       |
|--------|-------------------------------------|-------------------------------------|------|-------|-------|
| WMS163 | 5' ACC TCG ACA GAC CTG GTA CG 3'    | 5' GTC TTT GTC ACC CGA TGG AC 3'    | 127  | CT    | 55 °C |
| WMS164 | 5' ACA TTT CTC CCC CAT CGT C 3'     | 5' TTG TAA ACA AAT CGC ATG CG 3'    | 120  | CT    | 55 °C |
| WMS165 | 5' TGC AGT GGT CAG ATG TTT CC 3'    | 5' CTT TTC TTT CAG ATT GCG CC 3'    | 199  | GA    | 60 °C |
| WMS169 | 5' ACC ACT GCA GAG AAC ACA TAC G 3' | 5' GTG CTC TGC TCT AAG TGT GGG 3'   | 196  | GA    | 60 °C |
| WMS174 | 5' GGG TTC CTA TCT GGT AAA TCC C 3' | 5' GAC ACA CAT GTT CCT GCC AC 3'    | 173  | CT    | 55 °C |
| WMS179 | 5' AAG TTG AGT TGA TGC GGG AG 3'    | 5' CCA TGA CCA GCA TCC ACT C 3'     | 181  | GT    | 55 °C |
| WMS180 | 5' ATC CGC CTA AGG AAT AGT GT 3'    | 5' GAT CGC ACG GGA GAG AGA G 3'     | 84   | CT    | 50 °C |
| WMS181 | 5' TCA TTG GTA ATG AGG AGA GA 3'    | 5' GAA CCA TTC ATG TGC ATG TC 3'    | 135  | GA    | 50 °C |
| WMS182 | 5' TGA TGT AGT GAG CCC ATA GGC 3'   | 5' TTG CAC ACA GCC AAA TAA GG 3'    | 165  | CT    | 60 °C |
| WMS186 | 5' GCA GAG CCT GGT TCA AAA AG 3'    | 5' CGC CTC TAG CGA GAG CTA TG 5'    | 140  | GA    | 60 °C |
| WMS189 | 5' AGG AGC AGC GGA ACG AAC 3'       | 5' AGA AAT ACG GAA ACC CAC CC 3'    | 117  | CA    | 55 °C |
| WMS190 | 5' GTG CTT GCT GAG CTA TGA GTC 3'   | 5' GTG CCA CGT GGT ACC TTT G 3'     | >201 | CT,GT | 60 °C |
| WMS191 | 5' AGA CTG TTG TTT GCG GGC 3'       | 5' TAG CAC GAC AGT TGT ATG CAT G 3' | 128  | CT    | 60 °C |
| WMS192 | 5' GGT TTT CTT TCA GAT TGC GC 3'    | 5' CGT TGT CTA ATC TTG CCT TGC 3'   | 191  | CT    | 60 °C |
| WMS193 | 5' CTT TGT GCA CCT CTC TCT CC 3'    | 5' AAT TGT GTT GAT GAT TTG GGG 3'   | 171  | CT,CA | 60 °C |
| WMS194 | 5' GAT CTG CTC TAC TCT CCT CC 3'    | 5' CGA CGC AGA ACT TAA ACA AG 3'    | 131  | CT    | 50 °C |
| WMS195 | 5' AGG TGC CGT CGC GTC TAC 3'       | 5' ACC CCC CAC GTC AGA GAG 3'       | 108  | CT    | 60 °C |
| WMS197 | 5' GAG AAA GAG GTC TGG AGG TCG 3'   | 5' CAA AAT GCA CAA GAA TGG AGG 3'   | 126  | CT    | 60 °C |
| WMS198 | 5' TTG AAC CCG AAG GAG TAC AG 3'    | 5' TCA GTT TAT TTT GGG CAT GTG 3'   | 130  | CA    | 60 °C |
| WMS200 | 5' TCA ACG GAA CAG ATG AGC G 3'     | 5' GAC CTG ATG AGA GCA AGC AC 3'    | 250  | CT    | 60 °C |
| WMS203 | 5' CCC AAA GCA GCG CAA GC 3'        | 5' ACC AAT GCT ATC GGC TCG 3'       | 139  | CA,GA | 55 °C |
| WMS205 | 5' CGA CCC GGT TCA CTT CAG 3'       | 5' AGT CGC CGT TGT ATA GTG CC 3'    | 152  | CT    | 60 °C |
| WMS210 | 5' TGC ATC AAG AAT AGT GTG GAA G 3' | 5' TGA GAG GAA GGC TCA CAC CT 3'    | 192  | GA    | 60 °C |
| WMS212 | 5' AAG CAA CAT TTG CTG CAA TG 3'    | 5' TGC AGT TAA CTT GTT GAA AGG A 3' | 104  | CT    | 60 °C |
| WMS213 | 5' TGC CTG GCT CGT TCT ATC TC 3'    | 5' CTA GCT TAG CAC TGT CGC CC 3'    | 184  | GA    | 60 °C |
| WMS218 | 5' CGG CAA ACG GAT ATC GAC 3'       | 5' AAC AGT AAC TCT CGC CAT AGC C 3' | 149  | CT    | 60 °C |
| WMS219 | 5' GAT GAG CGA CAC CTA GCC TC 3'    | 5' GGG GTC CGA GTC CAC AAC 3'       | 181  | GAimp | 60 °C |
| WMS224 | 5' TGA GTC CAG CAC TGC TGC 3'       | 5' CAA CAT CCG CTC GTA TTC AA 3'    | 142  | CT    | 50 °C |

|        |                                      |                                      |            |       |
|--------|--------------------------------------|--------------------------------------|------------|-------|
| WMS228 | 5' TCA TAT GCA CCT CTT TCC TAG G 3'  | 5' GTG TGC CAC CTT TGA CGT C 3'      | CT,CA      | 60 °C |
| WMS231 | 5' AGC TCG GGA TGA AGC GTG 3'        | 5' GAT CCG CCG CTG CGT TT 3'         | GAimp      | 60 °C |
| WMS232 | 5' ATC TCA ACG GCA AGC CG 3'         | 5' CTG ATG CAA GCA ATC CAC C 3'      | GA         | 55 °C |
| WMS233 | 5' TCA AAA CAT AAA TGT TCA TTG GA 3' | 5' TCA ACC GTG TGT AAT TTT GTC C 3'  | CT         | 60 °C |
| WMS234 | 5' GAG TCC TGA TGT GAA GCT GTT G 3'  | 5' CTC ATT GGG GTG TGT ACG TG 3'     | CT,CA      | 55 °C |
| WMS237 | 5' GAA TCA CTT GTG AAG CAT CTG G 3'  | 5' CTG GAT GCA TCA CAT CCA AC 3'     | CT         | 55 °C |
| WMS238 | 5' TCG CTT CTA CCG CTC ACC 3'        | 5' AGT GCC TTG CCG AGG TC 3'         | CT,GT,GGGT | 55 °C |
| WMS241 | 5' TCT TCC AAC TAA AGC ATA GC 3'     | 5' CTT CCA TGG ACT ACA TAC TAG C 3'  | GA         | 55 °C |
| WMS242 | 5' TCC AAG GCA GTA GGC AGG 3'        | 5' TGT TGT TGG CCT GTA TGC AT 3'     | GA         | 55 °C |
| WMS244 | 5' GGC AGC TGA GGC AAT CTG 3'        | 5' TTT GGA CAT TTC CCA GCG 3'        | CAimp      | 60 °C |
| WMS245 | 5' CAG CGC AGT TAG CTC GC 3'         | 5' ATC TGT CCA TTC GAG CGC 3'        | CT         | 60 °C |
| WMS247 | 5' GCA ATC TTT TTT CTG ACC ACG 3'    | 5' ATG TGC ATG TCG GAC GC 3'         | GA         | 60 °C |
| WMS248 | 5' AGG ACT TCC GCA CCC TG 3'         | 5' TGG CGT GGT CTA AAT GGA C 3'      | CA         | 60 °C |
| WMS249 | 5' CAA ATG GAT CGA GAA AGG GA 3'     | 5' CTG CCA TTT TTC TGG ATC TAC C 3'  | GAimp      | 60 °C |
| WMS251 | 5' CAA CTG GTT GCT ACA CAA GCA 3'    | 5' GGG ATG TCT GTT CCA TCT TAG 3'    | CA         | 55 °C |
| WMS255 | 5' CAA CTG TAC GTA GGT TTC AIT GC 3' | 5' TCT GCC GTA AGT CGC CTC 3'        | GA         | 55 °C |
| WMS257 | 5' AGA GTG CAT GGT GGG ACG 3'        | 5' CCA AGA CGA TGC TGA AGT CA 3'     | GT         | 60 °C |
| WMS258 | 5' GAT CGC TTC ATC TCT CTC TCT C 3'  | 5' GTA CAC GCC GTA GGC CC 3'         | CT         | 60 °C |
| WMS259 | 5' AGG GAA AAG ACA TCT TTT TTT TC 3' | 5' CGA CCG ACT TCG GGT TC 3'         | GA         | 55 °C |
| WMS260 | 5' GCC CCC TTG CAC AAA TC 3'         | 5' CGC AGC TAC AGG AGG CC 3'         | GA         | 55 °C |
| WMS261 | 5' CTC CCT GTA CGC CTA AGG C 3'      | 5' CTC GCG CTA CTA GCC ATT G 3'      | CT         | 55 °C |
| WMS263 | 5' TCT GCC GTA AGT CGC CTC 3'        | 5' GGT TTC ATT GCT TGC CCT AA 3'     | CT         | 55 °C |
| WMS264 | 5' GAG AAA CAT GCC GAA CAA CA 3'     | 5' GCA TGC ATG AGA ATA GGA ACT G 3'  | CA         | 60 °C |
| WMS265 | 5' TGT TGC GGA TGG TCA CTA TT 3'     | 5' GAG TAC ACA TTT GGC CTC TGC 3'    | GT         | 55 °C |
| WMS268 | 5' AGG GGA TAT GTT GTC ACT CCA 3'    | 5' TTA TGT GAT TGC GTA CGT ACC C 3'  | GAimp      | 55 °C |
| WMS269 | 5' TGC ATA TAA ACA GTC ACA CAC CC 3' | 5' TTT GAG CTC CAA AGT GAG TTA GC 3' | CA         | 60 °C |
| WMS271 | 5' CAA GAT CGT GGA GCC AGC 3'        | 5' AGC TGC TAG CTT TTG GGA CA 3'     | CT,GA      | 60 °C |
| WMS272 | 5' TGC TCT TTG GCG AAT ATA TGG 3'    | 5' GTT CAA AAC AAA TTA AAA GGC CC 3' | CA         | 55 °C |

|        |                                      |                                     |           |            |       |
|--------|--------------------------------------|-------------------------------------|-----------|------------|-------|
| WMS273 | 5' ATT GGA CGG ACA GAT GCT TT 3'     | 5' AGC AGT GAG GAA GGG GAT C 3'     | 167       | GA         | 55 °C |
| WMS274 | 5' AAC TTG CAA AAC TGT TCT GA 3'     | 5' TAT TTG AAG CGG TTT GAT TT 3'    | 179       | GT         | 50 °C |
| WMS275 | 5' AAT TTT CTT CCT CAC TTA TTC T 3'  | 5' AAC AAA AAA TTA GGG CC 3'        | 107       | CT         | 50 °C |
| WMS276 | 5' ATT TGC CTG AAG AAA ATA TT 3'     | 5' AAT TTC ACT GCA TAC ACA AG 3'    | 99        | CT         | 55 °C |
| WMS278 | 5' GTT GCT TCA TGA ACG CTC AA 3'     | 5' CTG CCC AAT TTT CTC CAC TC 3'    | 241       | GTimpGAimp | 55 °C |
| WMS281 | 5' CGG CCA TAT TTC TGT AAG TAT GC 3' | 5' GCA GGT AAT GGC CGG AC 3'        | 135       | GT         | 60 °C |
| WMS282 | 5' TTG GCC GTG TAA GGC AG 3'         | 5' TCT CAT TCA CAC ACA CTA GC 3'    | 220       | GA         | 55 °C |
| WMS284 | 5' AAT GAA AAA ACA CTT GCG TGG 3'    | 5' GCA CAT TTT TCA CTT TCG GG 3'    | 123       | GA         | 60 °C |
| WMS285 | 5' ATG ACC CTT CTG CCA AAC AC 3'     | 5' ATC GAC CGG GAT CTA GCC 3'       | 243       | GA         | 60 °C |
| WMS291 | 5' CAT CCC TAC GCC ACT CTG C 3'      | 5' AAT GGT ATC TAT TCC GAC CCG 3'   | > 158     | CA         | 60 °C |
| WMS292 | 5' TCA CCG TGG TCA CCG AC 3'         | 5' CCA CCG AGC CGA TAA TGT AC 3'    | 220       | CT         | 60 °C |
| WMS293 | 5' TAC TGG TTC ACA TTG GTG CG 3'     | 5' TCG CCA TCA CTC GTT CAA G 3'     | 201       | CA         | 55 °C |
| WMS294 | 5' GGA TTG GAG TTA AGA GAG AAC CG 3' | 5' GCA GAG TGA TCA ATG CCA GA 3'    | 100       | GAimp      | 55 °C |
| WMS295 | 5' GTG AAG CAG ACC CAC AAC AC 3'     | 5' GAC GGC TGC GAC GTA GAG 3'       | 258       | GA         | 60 °C |
| WMS296 | 5' AAT TCA ACC TAC CAA TCT CTG 3'    | 5' GCC TAA TAA ACT GAA AAC GAG 3'   | 149       | CT         | 55 °C |
| WMS297 | 5' ATC GTC ACG TAT TTT GCA ATG 3'    | 5' TGC GTA AGT CTA GCA TTT TCT G 3' | 150       | GT, GA     | 55 °C |
| WMS299 | 5' ACT ACT TAG GCC TCC CGC C 3'      | 5' TGA CCC ACT TGC AAT TCA TC 3'    | 208       | GA, TAG    | 55 °C |
| WMS301 | 5' GAG GAG TAA GAC ACA TGC CC 3'     | 5' GTG GCT GGA GAT TCA GGT TC 3'    | 204       | GA, G      | 55 °C |
| WMS302 | 5' GCA AGA AGC AAC AGC AGT AAC 3'    | 5' CAG ATG CTC TTC TCT GCT GG 3'    | 180 (340) | GA         | 60 °C |
| WMS304 | 5' AGG AAA CAG AAA TAT CGC GG 3'     | 5' AGG ACT GTG GGG AAT GAA TG 3'    | 217       | CT         | 55 °C |
| WMS311 | 5' TCA CGT GGA AGA CGC TCC 3'        | 5' CTA CGT GCA CCA CCA TTT TG 3'    | 151       | GA         | 60 °C |
| WMS312 | 5' ATC GCA TGA TGC ACG TAG AG 3'     | 5' ACA TGC ATG CCT ACC TAA TGG 3'   | 235       | GA         | 60 °C |
| WMS313 | 5' CCG CCC TCA TTA AGT TTC AC 3'     | 5' TTT GAC AAG TAC ACG AGT CTG C 3' | 156       | CT, GT     | 55 °C |
| WMS314 | 5' AGG AGC TCC TCT GTG CCA C 3'      | 5' TTC GGG ACT CTC TTC CCT G 3'     | 170       | CT         | 55 °C |
| WMS316 | 5' CAT GGA CAT TTT ACC ACA AGA C 3'  | 5' TGC GTG TGG TCC ACC TC 3'        | 176       | AT, GT     | 55 °C |
| WMS319 | 5' GGT TGC TGT ACA AGT GTT CAC G 3'  | 5' CGG GTG CTG TGT GTA ATG AC 3'    | 200       | CT         | 55 °C |
| WMS320 | 5' CGA GAT ACT ATG GAA GGT GAG G 3'  | 5' ATC TTT GCA AGG ATT GCC C 3'     | > 263     | GT, GA     | 55 °C |
| WMS321 | 5' CAA TGT GGA GAC GGT GTG C 3'      | 5' TGT TGC ATG CGA TCA TGC 3'       | 265       | GT, GAimp  | 60 °C |

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|--------|--------------------------------------|-----------|--------------|-------|
| WMS322 | 5' TCA CAA AAT GAT TTC TCA TCC G 3'  | 119       | GA           | 55 °C |
| WMS325 | 5' TTT CTT CTG TCG TTC TCT TCC C 3'  | 131       | CT           | 55 °C |
| WMS328 | 5' GCA ATC CAC GAG AAG AGA GG 3'     | 193       | GT           | 55 °C |
| WMS330 | 5' TTG CTA TCC ATG TGC CAG AG 3'     | 165       | GTT          | 55 °C |
| WMS332 | 5' AGC CAG CAA GTC ACC AAA AC 3'     | 231       | GA           | 60 °C |
| WMS333 | 5' GCC CGG TCA TGT AAA ACG 3'        | 150       | GA           | 55 °C |
| WMS334 | 5' AAT TTC AAA AAG GAG AGA GA 3'     | 123       | GA           | 50 °C |
| WMS335 | 5' CGT ACT CCA CTC CAC ACG G 3'      | 187 (225) | GA, GCGT     | 55 °C |
| WMS336 | 5' CCC TTT AAT CTC GCT CCC TC 3'     | 108       | CT           | 55 °C |
| WMS337 | 5' CCT CTT CCT CCC TCA CTT AGC 3'    | 183       | CT, CACT, CA | 55 °C |
| WMS339 | 5' AAT TTT CTT CCT CAC TTA TT 3'     | 159       | CT           | 50 °C |
| WMS340 | 5' GCA ATC TTT TTT CTG ACC ACG 3'    | 132       | GA           | 60 °C |
| WMS341 | 5' TTC AGT GGT AGC GGT CGA G 3'      | 133 (150) | CT           | 55 °C |
| WMS342 | 5' TAT CCA GAG CAG ACG GAC G 3'      | 169       | GT           | 55 °C |
| WMS344 | 5' CAA GGA AAT AGG CGG TAA CT 3'     | 131       | GT           | 55 °C |
| WMS346 | 5' CAA GCA AGG TTT CGT TTT ATC C 3'  | 203       | AT, GT       | 55 °C |
| WMS349 | 5' GGC TTC CAG AAA ACA ACA GG 3'     | 230       | GA           | 55 °C |
| WMS350 | 5' ACC TCA TCC ACA TGT TCT ACG 3'    | 146       | GT           | 55 °C |
| WMS353 | 5' CCA TGT TGA GTA GGT TCA GCC 3'    | 179       | GCGT, GT     | 60 °C |
| WMS356 | 5' AGC GTT CTT GGG AAT TAG AGA 3'    | 224       | GA           | 55 °C |
| WMS357 | 5' TAT GGT CAA AGT TGG ACC TCG 3'    | 123       | GA           | 55 °C |
| WMS358 | 5' AAA CAG CGG ATT TCA TCG AG 3'     | 164       | GAimp        | 55 °C |
| WMS359 | 5' CTA ATT GCA ACA GGT CAT GGG 3'    | 217       | CT, CTimp    | 55 °C |
| WMS361 | 5' GTA ACT TGT TGC CAA AGG GG 3'     | 126       | GAimp        | 60 °C |
| WMS368 | 5' CCA TTT CAC CTA ATG CCT GC 3'     | 249       | AT           | 60 °C |
| WMS369 | 5' CTG CAG GCC ATG ATG ATG 3'        | 188       | CTimp        | 60 °C |
| WMS371 | 5' GAC CAA GAT ATT CAA ACT GGC C 3'  | 170       | CA, GA       | 60 °C |
| WMS372 | 5' AAT AGA GCC CTG GGA CTG GG 3'     | >329      | GA           | 60 °C |
|        | 5' TGC AGA AAA CCA ACA AGG G 3'      |           |              |       |
|        | 5' TTT TTA CGC GTC AAC GAC G 3'      |           |              |       |
|        | 5' CAC AAA CTC TTG ACA TGT GCG 3'    |           |              |       |
|        | 5' ACA TGT TTC ATG CAG GTA GCC 3'    |           |              |       |
|        | 5' AGT GCT GGA AAG AGT AGT GAA GC 3' |           |              |       |
|        | 5' TTT CAG TTT GCG TTA AGC TTT G 3'  |           |              |       |
|        | 5' AAC ATG TGT TTT TAG CTA TC 3'     |           |              |       |
|        | 5' CGG TCC AAG TGC TAC CTT TC 3'     |           |              |       |
|        | 5' GTC TCT TTC TCG TAC TTC CAG G 3'  |           |              |       |
|        | 5' TGC TAA CTG GCC TTT GCC 3'        |           |              |       |
|        | 5' AAA CGA ACA ACC ACT CAA TC 3'     |           |              |       |
|        | 5' ACG AGG CAA GAA CAC ACA TG 3'     |           |              |       |
|        | 5' CCG ACA TCT CAT GGA TCC AC 3'     |           |              |       |
|        | 5' GGT CTA GCT TCG ACG ACA CC 3'     |           |              |       |
|        | 5' ATT TGA GTC TGA AGT TTG CA 3'     |           |              |       |
|        | 5' GCA TGT GGT CCA TGT ACT GC 3'     |           |              |       |
|        | 5' ATC GGT GCG TAC CAT CCT AC 3'     |           |              |       |
|        | 5' GCA TGG ATA GGA CGC CC 3'         |           |              |       |
|        | 5' CTT GGC CAG AAG CTA CGA AC 3'     |           |              |       |
|        | 5' CCA ATC AGC CTG CAA CAA C 3'      |           |              |       |
|        | 5' AGG CTG CAG CTC TTC TTC AG 3'     |           |              |       |
|        | 5' TCC GCT GTT GTT CTG ATC TC 3'     |           |              |       |
|        | 5' TAC TTG TGT TCT GGG ACA ATG G 3'  |           |              |       |
|        | 5' ACA AAG TGG CAA AAG GAG ACA 3'    |           |              |       |
|        | 5' AAT AAA ACC ATG AGC TCA CTT GC 3' |           |              |       |
|        | 5' ACC GTG GGT GTT GTG AGC 3'        |           |              |       |
|        | 5' AGC TCA GCT TGC TTG GTA CC 3'     |           |              |       |
|        | 5' GAA GGA CGA CAT TCC ACC TG 3'     |           |              |       |



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|--------|--------------------------------------|-------------------------------------|-----------|------------|-------|
| WMS374 | 5' ATA GTG TGT TGC ATG CTG TGT G 3'  | 5' TCT AAT TAG CGT TGG CTG CC 3'    | 213       | GT         | 60 °C |
| WMS375 | 5' ATT GGC GAC TCT AGC ATA TAC G 3'  | 5' GGG ATG TCT GTT CCA TCT TAG C 3' | 156       | CA         | 55 °C |
| WMS376 | 5' GGG CTA GAA AAC AGG AAG GC 3'     | 5' TCT CCC GGA GGG TAG GAG 3'       | 147       | CA, GAimp  | 60 °C |
| WMS382 | 5' GTC AGA TAA CGC CGT CCA AT 3'     | 5' CTA CGT GCA CCA CCA TTT TG 3'    | 115       | GA         | 60 °C |
| WMS383 | 5' ACG CCA GTT GAT CCG TAA AC 3'     | 5' GAC ATC AAT AAC CGT GGA TGG 3'   | 195       | GT         | 60 °C |
| WMS384 | 5' TTT TCA TTG TGC CCT CTA CT 3'     | 5' GCC AAG TTT CTT AGC TAG TTA A 3' | 204       | GTimp      | 55 °C |
| WMS388 | 5' CTA CAA TTC GAA GGA GAG GGG 3'    | 5' CAC CGC GTC AAC TAC TTA AGC 3'   | 162       | CT, CA, CA | 60 °C |
| WMS389 | 5' ATC ATG TCG ATC TCC TTG ACG 3'    | 5' TGC CAT GCA CAT TAG CAG AT 3'    | 130       | CT, GT     | 60 °C |
| WMS390 | 5' AAG TTT CAC ACA AGA TCT CTC C 3'  | 5' TGA CAA GTA CAC GAG TCT GC 3'    | 143       | CT, GT     | 55 °C |
| WMS391 | 5' ATA GCG AAG TCT CCC TAC TCC A 3'  | 5' ATG TGC ATG TCG GAC GC 3'        | 150       | CA, GA     | 55 °C |
| WMS393 | 5' TCA TCT GCT ATT TGT GCT ACA 3'    | 5' TCA AAT ACA CCA ATG TGC C 3'     | 107       | CA         | 55 °C |
| WMS395 | 5' TAC AAC CGC AAG TAA TGC CA 3'     | 5' TAC CAA CAC CCT AGC CCT TG 3'    | 147       | CA         | 60 °C |
| WMS397 | 5' TGT CAT GGA TTA TTT GGT CGG 3'    | 5' CTG CAC TCT CGG TAT ACC AGC 3'   | 179       | CT         | 55 °C |
| WMS400 | 5' GTG CTG CCA CCA CTT GC 3'         | 5' TGT AGG CAC TGC TTG GGA G 3'     | 139       | CA         | 60 °C |
| WMS403 | 5' CGA CAT TGG CTT CGG TG 3'         | 5' ATA AAA CAG TGC GGT CCA GG 3'    | 133       | CA         | 55 °C |
| WMS408 | 5' TCG ATT TAT TTG GGC CAC TG 3'     | 5' GTA TAA TTC GTT CAC AGC ACG C 3' | 176       | CA         | 55 °C |
| WMS410 | 5' GCT TGA GAC CGG CAC AGT 3'        | 5' CGA GAC CTT GAG GGT CTA GA 3'    | 334       | CA         | 55 °C |
| WMS411 | 5' CCC ATA CGA TGA TGT GTT TCC 3'    | 5' CAA ACG GAA CAT GGT CCC 3'       | 148       | CT         | 55 °C |
| WMS412 | 5' ATC AAC AAG GTT TGT GTG TTG G 3'  | 5' ATG AAA CGC GAC CTC CC 3'        | 121       | GA         | 55 °C |
| WMS413 | 5' TGC TTG TCT AGA TTG CTT GGG 3'    | 5' GAT CGT CTC GTC CTT GGC A 3'     | 94        | GA         | 60 °C |
| WMS415 | 5' GAT CTC CCA TGT CCG CC 3'         | 5' CGA CAG TCG TCA CTT GCC TA 3'    | 131       | GAimp      | 55 °C |
| WMS425 | 5' GAG CCC ACA AGC TGG CA 3'         | 5' TCG TTC TCC CAA GGC TTG 3'       | >143      | CT         | 60 °C |
| WMS427 | 5' AAA CTT AGA ACT GTA ATT TCA GA 3' | 5' AGT GTG TTC ATT TGA CAG TT 3'    | 215       | CA         | 50 °C |
| WMS428 | 5' CGA GGC AGC GAG GAT TT 3'         | 5' TTC TCC ACT AGC CCC GC 3'        | 143       | GA         | 60 °C |
| WMS429 | 5' TTG TAC ATT AAG TTC CCA TTA 3'    | 5' TTT AAG GAC CTA CAT GAC AC 3'    | 221 (290) | CT         | 50 °C |
| WMS434 | 5' ATG AGT TCC GCC AAA GAA TG 3'     | 5' ACG AAA TAC ACA AGT GGG ACA 3'   | 216       | GT         | 55 °C |
| WMS437 | 5' GAT CAA GAC TTT TGT ATC TCT C 3'  | 5' GAT GTC CAA CAG TTA GCT TA 3'    | 109       | CT         | 50 °C |
| WMS440 | 5' CCT ATG GTC TCC ATC ATG AGG 3'    | 5' TCA TGT CAA CTC AAG AAC ACG 3'   | 112       | CT         | 55 °C |

|        |                                     |      |                                     |        |       |
|--------|-------------------------------------|------|-------------------------------------|--------|-------|
| WMS443 | 5' GGG TCT TCA TCC GGA ACT CT 3'    | 134  | 5' CCA TGA TTT ATA AAT TCC ACC 3'   | CA, GA | 55 °C |
| WMS445 | 5' TTT GTT GGG GGT TAG GAT TAG 3'   | 192  | 5' CCT TAA CAC TTG CTG GTA GTG A 3' | CT     | 55 °C |
| WMS448 | 5' AAA CCA TAT TGG GAG GAA AGG 3'   | 231  | 5' CAC ATG GCA TCA CAT TTG TG 3'    | GA     | 60 °C |
| WMS455 | 5' ATT CGG TTC GCT AGC TAC CA 3'    | 151  | 5' ACG GAG AGC AAC CTG CC 3'        | GTimp  | 55 °C |
| WMS456 | 5' TCT GAA CAT TAC ACA ACC CTG A 3' | 132  | 5' TGC TCT CTC TGA ACC TGA AGC 3'   | GA     | 55 °C |
| WMS458 | 5' AAT GGC AAT TGG AAG ACA TAG C 3' | 113  | 5' TTC GCA ATG TTG ATT TGG C 3'     | CA     | 60 °C |
| WMS459 | 5' ATG GAG TGG TCA CAC TTT GAA 3'   | >138 | 5' AGC TTC TCT GAC CAA CTT CTC G 3' | GA     | 55 °C |
| WMS469 | 5' CAA CTC AGT GCT CAC ACA ACG 3'   | >156 | 5' CGA TAA CCA CTC ATC CAC ACC 3'   | CT     | 60 °C |
| WMS471 | 5' CGG CCC TAT CAT GGC TG 3'        | 149  | 5' GCT TGC AAG TTC CAT TTT GC 3'    | CA     | 60 °C |
| WMS473 | 5' TCA TAC GGG TAT GGT TGG AC 3'    | 220  | 5' CAC CCC CTT GTT GGT CAC 3'       | GTimp  | 55 °C |
| WMS476 | 5' ATG GGT TCG TAC TAA CAT CAG C 3' | >194 | 5' TTG CTG GTA GCT TCA ATC CC 3'    | GAimp  | 60 °C |
| WMS480 | 5' TGC TGC TAC TTG TAC AGA GGA C 3' | 188  | 5' CCG AAT TGT CCG CCA TAG 3'       | CT, CA | 60 °C |
| WMS484 | 5' ACA TCG CTC TTC ACA AAC CC 3'    | 145  | 5' AGT TCC GGT CAT GGC TAG G 3'     | CT     | 55 °C |
| WMS494 | 5' ATT GAA CAG GAA GAC ATC AGG G 3' | 198  | 5' TTC CTG GAG CTG TCT GGC 3'       | CA     | 60 °C |
| WMS495 | 5' GAG AGC CTC GCG AAA TAT AGG 3'   | 168  | 5' TGC TTC TGG TGT TCC TTC G 3'     | GA     | 60 °C |
| WMS497 | 5' GTA GTG AAG ACA AGG GCA TT 3'    | >106 | 5' CCG AAA GTT GGG TGA TAT AC 3'    | GTimp  | 55 °C |
| WMS499 | 5' ACT TGT ATG CTC CAT TGA TTG G 3' | 145  | 5' GGG GAG TGG AAA CTG CAT AA 3'    | GA     | 60 °C |
| WMS501 | 5' GGC TAT CTC TGG CGC TAA AA 3'    | 172  | 5' TCC ACA AAC AAG TAG CGC C 3'     | CA     | 60 °C |
| WMS512 | 5' AGC CAC CAT CAG CAA AAA TT 3'    | 185  | 5' GAA CAT GAG CAG TTT GGC AC 3'    | GT     | 60 °C |
| WMS513 | 5' ATC CGT AGC ACC TAC TGG TCA 3'   | 144  | 5' GGT CTG TTC ATG CCA CAT TG 3'    | CA     | 60 °C |
| WMS515 | 5' AAC ACA ATG GCA AAT GCA GA 3'    | 134  | 5' CCT TCC TAG TAA GTG TGC CTC A 3' | GTimp  | 60 °C |
| WMS518 | 5' AAT CAC AAC AAG GCG TGA CA 3'    | 166  | 5' CAG GGT GGT GCA TGC AT 3'        | CA     | 55 °C |
| WMS530 | 5' AAA TAG GAC AAC CCA CGG C 3'     | 186  | 5' TCA ACT TCT TGG CCT CCA TC 3'    | CT     | 55 °C |
| WMS532 | 5' ACT GCG TGT GCC TAC AAT TG 3'    | 142  | 5' TCA CTC GCA CTC GAT AGG C 3'     | GT     | 60 °C |
| WMS533 | 5' AAG GCG AAT CAA ACG GAA TA 3'    | 147  | 5' GTT GCT TTA GGG GAA AAG CC 3'    | CT, CA | 60 °C |
| WMS537 | 5' ACA TAA TGC TTC CTG TGC ACC 3'   | 209  | 5' GCC ACT TTT GTG TCG TTC CT 3'    | CA, TA | 60 °C |
| WMS538 | 5' GCA TTT CGG GTG AAC CC 3'        | 147  | 5' GTT GCA TGT ATA CGT TAA GCG G 3' | GTimp  | 60 °C |
| WMS540 | 5' TCT CGC TGT GAA ATC CTA TTT C 3' | 129  | 5' AGG CAT GGA TAG AGG GGC 3'       | CTimp  | 55 °C |

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|        |                                     |                                     |     |              |       |
|--------|-------------------------------------|-------------------------------------|-----|--------------|-------|
| WMS544 | 5' TAG AAT TCT TTA TGG GGT CTG C 3' | 5' AGG ATT CCA ATC CTT CAA AAT T 3' | 167 | CT, ATCT, CT | 55 °C |
| WMS550 | 5' CCC ACA AGA ACC TTT GAA GA 3'    | 5' CAT TGT GTG TGC AAG GCA C 3'     | 150 | CT, GT       | 55 °C |
| WMS554 | 5' TGC CCA CAA CGG AAC TTG 3'       | 5' GCA ACC ACC AAG CAC AAA GT 3'    | 160 | CT, GTimp    | 60 °C |
| WMS565 | 5' GCG TCA GAT ATG CCT ACC TAG G 3' | 5' AGT GAG TTA GCC CTG AGC CA 3'    | 142 | CA           | 60 °C |
| WMS566 | 5' TCT GTC TAC CCA TGG GAT TTG 3'   | 5' CTG GCT TCG AGG TAA GCA AC 3'    | 130 | CA, TA       | 60 °C |
| WMS569 | 5' GGA AAC TTA TTG ATT GAA AT 3'    | 5' TCA ATT TTG ACA GAA GAA TT 3'    | 134 | GT           | 47 °C |
| WMS570 | 5' TCG CCT TTT ACA GTC GGC 3'       | 5' ATG GGT AGC TGA GAG CCA AA 3'    | 143 | CT, GT       | 60 °C |
| WMS573 | 5' AAG AGA TAA CAT GCA AGA AA 3'    | 5' TTC AAA TAT GTG GGA ACT AC 3'    | 212 | CA           | 50 °C |
| WMS577 | 5' ATG GCA TAA TTT GGT GAA ATT G 3' | 5' TGT TTC AAG CCC AAC TTC TAT T 3' | 133 | CA, TA       | 55 °C |
| WMS582 | 5' AAG CAC TAC GAA AAT ATG AC 3'    | 5' TCT TAA GGG GTG TTA TCA TA 3'    | 151 | CA           | 50 °C |
| WMS583 | 5' TTC ACA CCC AAC CAA TAG CA 3'    | 5' TCT AGG CAG ACA CAT GCC TG 3'    | 165 | CA           | 60 °C |
| WMS588 | 5' GAT CCC CAA TTG CAT GTT G 3'     | 5' CTT GCA ACT GGG GGA CAC 3'       | 102 | GT           | 60 °C |

\* 'CS' Weizensorte 'Chinse Spring'

These markers are distinguished by a high degree of polymorphism between different wheat varieties or lines and usually detect several alleles per genetic locus in different wheat lines.

They can therefore be used for DNA fingerprinting, species identification, relationship or similarity studies, characterization of cytological lines, such as deletion lines, substitution lines, addition lines, etc. and all forms of genetic mappings, including mapping of individual genes and quantitative distinguishing features (QTLs). In addition, their use is also very suitable for automation and it is possible to carry out the detection of the products with nonradioactive methods.

With the help of this inventive marker, the possibility is provided, for example, of differentiating almost all European wheat lines.

The invention is described in greater detail below by means of examples.

### **1. Amplification of the Microsatellite Markers**

The microsatellite markers are amplified according to the following protocol:

10 mM tris-HCl, pH 8

50 mM KCl

1.5 mM MgCl<sub>2</sub> (in a few exceptional cases 3 mM MgCl<sub>2</sub>)

0.01% (w/v) gelatin

0.2 mM of each desoxynucleotide

250 nM of each primer (in each case to the left and right of a pair)

1 - 2 units taq polymerase

50 - 150 ng matrixes (template) DNA

are amplified in a volume of 25 or 50  $\mu$ L according to the following profile:

|      |                              |           |
|------|------------------------------|-----------|
| 92°C | 3 minute                     |           |
| 92°C | 1 minute (denaturing phase)  |           |
| 60°C | 1 minutes (annealing phase)  | 45 cycles |
| 72°C | 2 minutes (elongation phase) |           |
| 72°C | 10 minutes (extension phase) |           |

The amplification takes place in a Perkin Elmer 9600 with lid heating or in an MJ Research Thermocycler without lid heating. In this apparatus, a layer of mineral oil is placed over the reactions. The temperature of the annealing phase depends on the melting point ( $T_m$ ) of the primer and in some cases even is 50°C or 55°C.

## **2. Separation of the Microsatellite Markers on Polyacrylamide Gels, Which Are Not Denaturing**

The PCR reactions are mixed with 1/10 volume of stop buffer (0.02 M tris acetate of pH 8.1, 0.025 M sodium acetate, 0.02 M EDTA, 70% glycerin, 0.2% SDS, 0.6% bromphenol blue, 0.6% xylene cyanol) and in each case 25  $\mu$ L are separated in 10% polyacrylamide gels (1.5 mm thick, 18 cm long).

Formulation for polyacrylamide gel (10%):

25 mL stock acrylamide solution (19 g acrylamide, 1 g bisacrylamide, diluted to 100 mL with water)

10 mL 5X TBE (1X TBE = 0.09 M tris borate of pH 8.3, 0.002 M EDTA)  
15 mL water

are mixed and the polymerization is started by the addition of 220  $\mu$ L of ammonium persulfate (10%, freshly prepared) and 20  $\mu$ L of TEMED. Immediately after the addition, the mixture is poured into the sealed gel mold and the comb for forming pockets is inserted. The polymerization is completed after about 1 hour. The gel is placed in the gel chamber and a preliminary run is carried out without samples for about 30 minutes at 150 volts in 1X TBE. After that, the samples are loaded (25  $\mu$ L of each) and the separation is carried out for 14 - 16 hours at 100 volts.

After the electrophoresis is completed, the gel is stained for about minutes in ethidium bromide (1 - 2 drops of 10 mg/mL in 1 liter of water) and the fragments are made visible by a UV transilluminator and documented.

### 3. Separation of Microsatellite Markers on Denaturing Gels

For the separation of the amplified fragments on denaturing gels, an automatic laser fluorescence (A.L.F.) sequencer (Pharmacia), for example, is used. In order to enable the fragments to be detected by means of a laser, one primer per pair is marked at the 5' end with fluorescein. Per PCR reaction, 0.3 to 1.5 microliters are mixed with 2.5 microliters of stop buffer (deionized formamide; 5 mg/mL dextran blue), denatured (1 minute; 90°C) and loaded onto the gel. Gel plates with a 9 cm separation distance are used, as recommended by the manufacturer for the fragment analysis. The gel solution contains 6.5% Long-Ranger (AT Biochem), 7M urea and 1.2X TBE buffer. The gels are 0.35 or 0.5 mm thick. The conditions for the gel run are 600 V, 40 mA, 50 W, 0.84 s data collection interval and 2 mW laser energy. The gel runs are ended after about 80 to 90 minutes. This is sufficient for detecting fragments up to a size of 300 bp. A gel can be used for four or five runs. For each gel

run, a data set is obtained. With this data set and by means of internal size standards, the exact fragment sizes are determined in the computer program Fragment Manager (Pharmacia) and thus the smallest size differences of a base pair are determined.

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**Claims**

1. Microsatellite markers (based on hypervariable genome sections) for plants of the *Triticum aestivum* species, as well as of the Tribe Triticeae using the polymerase chain reaction (PCR), characterized in that a sequence tagged site (STS), which is defined by two specific primers, which average a length of  $20 \pm 3$  bases and flank a microsatellite sequence, which microsatellite markers are amplified to polymorphisms (PCR products of different length).

2. The microsatellite markers of claim 1, characterized in that the microsatellite sequence is a tandem-repetitive n-fold repetition of a di-, tri- or tetranucleotide sequence, in which  $n \geq 10$ .

3. The microsatellite markers of claim 1, characterized in that the microsatellite sequence is a composite microsatellite sequence.

4. The microsatellite markers of claim 1, characterized in that the microsatellite sequence is an imperfect sequence, in which individual bases are mutated.

5. The microsatellite markers of claim 1, characterized in that the following primer pairs with assigned microsatellite sequences or a number thereof are contained.



| WMS Number<br>WMS-Nummer | WMS Primer left<br>WMS Primer links | WMS Primer Right<br>WMS Primer rechts | Repeat Type<br>Repeat-Typ |
|--------------------------|-------------------------------------|---------------------------------------|---------------------------|
| WMS052                   | 5' CTA TGA GGC GGA GGT TGA AG 3'    | 5' TGC GGT GCT CTT CCA TTT 3'         | GTimp                     |
| WMS055                   | 5' GCA TCT GGT ACA CTA GCT GCC 3'   | 5' TCA TGG ATG CAT CAC ATC CT 3       | CTimp                     |
| WMS057                   | 5' TCG ATT CTG AAA GGT TCA TCG 3'   | 5' CGA TCA AGT AGT TGA AAG CGC 3'     | AAAAAimp                  |
| WMS058                   | 5' TCT GAT CCC GTG AGT GTA ACA 3'   | 5' GAA AAA AAT TGC ATA TGA GCC C 3'   | CA                        |
| WMS060                   | 5' TGT CCT ACA CGG ACC ACG T 3'     | 5' GCA TTG ACA GAT GCA CAC G 3'       | CA                        |
| WMS063                   | 5' TCG ACC TGA TCG CCC CTA 3'       | 5' CGC CCT GGG TGA TGA ATA GT 3'      | GAA,CA,TA                 |
| WMS067                   | 5' ACC ACA CAA ACA AGG TAA GCG 3'   | 5' CAA CCC TCT TAA TTT TGT TGG G 3'   | CA                        |
| WMS068                   | 5' AGG CCA GAA TCT GGG AAT G 3'     | 5' CTC CCT AGA TGG GAG AAG GG 3'      | GA                        |
| WMS070                   | 5' AGT GGC TGG GAG AGT GTC AT 3'    | 5' GCC CAT TAC CGA GGA CAC 3'         | GT                        |
| WMS071                   | 5' GGC AGA GCA GCG AGA CTC 3'       | 5' CAA GTG GAG CAT TAG GTA CAC G 3'   | GT                        |
| WMS077                   | 5' ACA AAG GTA AGC AGC ACC TG 3'    | 5' ACC CTC TTG CCC GTG TTG 3'         | CA,GA                     |
| WMS082                   | 5' ACG TTA GAA GGT GCA ATG GG 3'    | 5' AGT GGA TGC ACC GAC TTT G 3'       | GT,GAimp                  |
| WMS088                   | 5' CAC TAC AAC TAT GCG CTC GC 3'    | 5' TCC ATT GGC TTC TCT CTC AA 3'      | GT                        |
| WMS095                   | 5' GAT CAA ACA CAC ACC CCT CC 3'    | 5' AAT GCA AAG TGA AAA ACC CG 3'      | CA                        |
| WMS099                   | 5' AAG ATG GAC GTA TGC ATC ACA 3'   | 5' GCC ATA TTT GAT GAC GCA TA 3'      | CA                        |
| WMS102                   | 5' TCT CCC ATC CAA CGC CTC 3'       | 5' TGT TGG TGG CTT GAC TAT TG 3'      | CT                        |
| WMS106                   | 5' CTG TTC TTG CGT GGC ATT AA 3'    | 5' AAT AAG GAC ACA ATT GGG ATG G 3'   | GA                        |
| WMS107                   | 5' ATT AAT ACC TGA GGG AGG TGC 3'   | 5' GGT CTC AGG AGC AAG AAC AC 3'      | CT                        |
| WMS108                   | 5' CGA CAA TGG GGT CTT AGC AT 3'    | 5' TGC ACA CTT AAA TTA CAT CCG C 3'   | GTimp                     |
| WMS111                   | 5' TCT GTA GGC TCT CTC CGA CTG 3'   | 5' ACC TGA TCA GAT CCC ACT CG 3'      | CT,GT                     |
| WMS112                   | 5' CTA AAC ACG ACA GCG GTG G 3'     | 5' GAT ATG TGA GCA GCG GTC AG 3'      | CTimp                     |
| WMS113                   | 5' ATT CGA GGT TAG GAG GAA GAG G 3' | 5' GAG GGT CGG CCT ATA AGA CC 3'      | GT                        |
| WMS114                   | 5' ACA AAC AGA AAA TCA AA CCC G 3'  | 5' ATC CAT CGC CAT TGG AGT G 3'       | GA                        |
| WMS118                   | 5' GAT GTT GCC ACT TGA GCA TG 3'    | 5' GAT TAG TCA AAT GGA ACA CCC C 3'   | CA                        |
| WMS119                   | 5' TGA CTA ACA TCC TTT GTC ACG C 3' | 5' CAT GTC TCA ACC ACC CAC AG 3'      | GTimp                     |
| WMS120                   | 5' GAT CCA CCT TCC TCT CTC TC 3'    | 5' GAT TAT ACT GGT GCC GAA AC 3'      | CT,CA                     |
| WMS121                   | 5' TCC TCT ACA AAC AAA CAC AC 3'    | 5' CTC GCA ACT AGA GGT GTA TG 3'      | CA                        |

|        |                                     |                                     |          |
|--------|-------------------------------------|-------------------------------------|----------|
| WMS122 | 5' GGG TGG GAG AAA GGA GAT G 3'     | 5' AAA CCA TCC TCC ATC CTG G 3'     | CT,CA    |
| WMS124 | 5' GCC ATG GCT ATC ACC CAG 3'       | 5' ACT GTT CGG TGC AAT TTG AG 3'    | CT,GTimp |
| WMS126 | 5' CAC ACG CTC CAC CAT GAC 3'       | 5' GTT GAG TTG ATG CGG GAG G 3'     | CA       |
| WMS128 | 5' AGC ACA TTT TAA CAC AGA TA 3'    | 5' ATC TGT GAA ATT TTG AAA AC 3'    | CA       |
| WMS129 | 5' TCA GTG GGC AAG CTA CAC AG 3'    | 5' AAA ACT TAG TAG CCG CGT 3'       | GTimp    |
| WMS130 | 5' AGC TCT GCT TCA CGA GGA AG 3'    | 5' CTC CTC TTT ATA TCG CGT CCC 3'   | GT       |
| WMS131 | 5' AAT CCC CAC CGA TTC TTC TC 3'    | 5' AGT TCG TGG GTC TCT GAT GG 3'    | CT       |
| WMS132 | 5' TAC CAA ATC GAA ACA CAT CAG G 3' | 5' CAT ATC AAG GTC TCC TTC CCC 3'   | GA,GAA   |
| WMS133 | 5' ATC TAA ACA AGA CGG CGG TG 3'    | 5' ATC TGT GAC AAC CCG TGA GA 3'    | CT       |
| WMS134 | 5' CAT GGA ACT TAG ACA GAA TTG 3'   | 5' CAG TAC TTG GTA CTG AAC AGG 3'   | CA       |
| WMS135 | 5' TGT CAA CAT CGT TTT GAA AAG G 3' | 5' ACA CTG TCA ACC TGG CAA TG 3'    | GA       |
| WMS136 | 5' GAC AGC ACC TTG CCC TTT G 3'     | 5' CAT CGG CAA CAT GCT CAT C 3'     | CT       |
| WMS140 | 5' ATG GAG ATA TTT GGC CTA CAA C 3' | 5' CTT GAC TTC AAG GCG TGA CA 3'    | CT       |
| WMS144 | 5' TTT GCT GTG GTA CGA AAC ATA C 3' | 5' ACT CAC AAA TGT CTA ATA AAA C 3' | GT       |
| WMS146 | 5' CCA AAA AAA CTG CCT GCA TG 3'    | 5' CTC TGG CAT TGC TCC TTG G 3'     | GAimp    |
| WMS148 | 5' GTG AGG CAG CAA GAG AGA AA 3'    | 5' CAA AGC TTG ACT CAG ACC AAA 3'   | CA       |
| WMS149 | 5' CAT TGT TTT CTG CCT CTA GCC 3'   | 5' CTA GCA TCG AAC CTG AAC AAG 3'   | GA       |
| WMS153 | 5' GAT CTC GTC ACC CGG AAT TC 3'    | 5' TGG TAG AGA AGG ACG GAG AG 3'    | GA       |
| WMS154 | 5' TCA CAG AGA GAG AGG GAG GG 3'    | 5' ATG TGT ACA TGT TGC CTG CA 3'    | GA       |
| WMS155 | 5' CAA TCA TTT CCC CCT CCC 3'       | 5' AAT CAT TGG AAA TCC ATA TGC C 3' | CT       |
| WMS156 | 5' CCA ACC GTG CTA TTA GTC ATT C 3' | 5' CAA TGC AGG CCC TCC TAA C 3'     | GT       |
| WMS157 | 5' GTC GTC GCG GTA AGC TTG 3'       | 5' GAG TGA ACA CAC GAG GCT TG 3'    | CT       |
| WMS159 | 5' GGG CCA ACA CTG GAA CAC 3'       | 5' GCA GAA GCT TGT TGG TAG GC 3'    | GT       |
| WMS160 | 5' TTC AAT TCA GTC TTG GCT TGG 3'   | 5' CTG CAG GAA AAA AAG TAC ACC C 3' | GA       |
| WMS161 | 5' GAT CGA GTG ATG GCA GAT GG 3'    | 5' TGT GAA TTA CTT GGA CGT GG 3'    | CT       |
| WMS162 | 5' AGT GGA TCG ACA AGG CTC TG 3'    | 5' AGA AGA AGC AAA GCC TTC CC 3'    | CA       |
| WMS163 | 5' ACC TCG ACA GAC CTG GTA CG 3'    | 5' GTC TTT GTC ACC CGA TGG AC 3'    | CT       |
| WMS164 | 5' ACA TTT CTC CCC CAT CGT C 3'     | 5' TTG TAA ACA AAT CGC ATG CG 3'    | CT       |

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|        |                                     |                                     |       |
|--------|-------------------------------------|-------------------------------------|-------|
| WMS165 | 5' TGC AGT GGT CAG ATG TTT CC 3'    | 5' CTT TTC TTT CAG ATT GCG CC 3'    | GA    |
| WMS169 | 5' ACC ACT GCA GAG AAC ACA TAC G 3' | 5' GTG CTC TGC TCT AAG TGT GGG 3'   | GA    |
| WMS174 | 5' GGG TTC CTA TCT GGT AAA TCC C 3' | 5' GAC ACA CAT GTT CCT GCC AC 3'    | CT    |
| WMS179 | 5' AAG TTG AGT TGA TGC GGG AG 3'    | 5' CCA TGA CCA GCA TCC AC 3'        | GT    |
| WMS180 | 5' ATC CGC CTA AGG AAT AGT GT 3'    | 5' GAT CGC ACG GGA GAG AG,          | CT    |
| WMS181 | 5' TCA TTG GTA ATG AGG AGA GA 3'    | 5' GAA CCA TTC ATG TGC ATG TC 3'    | GA    |
| WMS182 | 5' TGA TGT AGT GAG CCC ATA GGC 3'   | 5' TTG CAC ACA GCC AAA TAA GG 3'    | CT    |
| WMS186 | 5' GCA GAG CCT GGT TCA AAA AG 3'    | 5' CGC CTC TAG CGA GAG CTA TG 5'    | GA    |
| WMS189 | 5' AGG AGC AGC GGA ACG AAC 3'       | 5' AGA AAT ACG GAA ACC CAC CC 3'    | CA    |
| WMS190 | 5' GTG CTT GCT GAG CTA TGA GTC 3'   | 5' GTG CCA CGT GGT ACC TTT G 3'     | CT,GT |
| WMS191 | 5' AGA CTG TTG TTT GCG GGC 3'       | 5' TAG CAC GAC AGT TGT ATG CAT G 3' | CT    |
| WMS192 | 5' GGT TTT CTT TCA GAT TGC GC 3'    | 5' CGT TGT CTA ATC TTG CCT TGC 3'   | CT    |
| WMS193 | 5' CTT TGT GCA CCT CTC TCT CC 3'    | 5' AAT TGT GTT GAT GAT TTG GGG 3'   | CT,CA |
| WMS194 | 5' GAT CTG CTC TAC TCT CCT CC 3'    | 5' CGA CGC AGA ACT TAA ACA AG 3'    | CT    |
| WMS195 | 5' AGG TGC CGT CGC GTC TAC 3'       | 5' ACC CCC CAC GTC AGA GAG 3'       | CT    |
| WMS197 | 5' GAG AAA GAG GTC TGG AGG TCG 3'   | 5' CAA AAT GCA CAA GAA TGG AGG 3'   | CT    |
| WMS198 | 5' TTG AAC CCG AAG GAG TAC AG 3'    | 5' TCA GTT TAT TTT GGG CAT GTG 3'   | CA    |
| WMS200 | 5' TCA ACG GAA CAG ATG AGC G 3'     | 5' GAC CTG ATG AGA GCA AGC AC 3'    | CT    |
| WMS203 | 5' CCC AAA GCA GCG CAA GC 3'        | 5' ACC AAT GCT ATC GGC TCG 3'       | CA,GA |
| WMS205 | 5' CGA CCC GGT TCA CTT CAG 3'       | 5' AGT CGC CGT TGT ATA GTG CC 3'    | CT    |
| WMS210 | 5' TGC ATC AAG AAT AGT GTG GAA G 3' | 5' TGA GAG GAA GGC TCA CAC CT 3'    | GA    |
| WMS212 | 5' AAG CAA CAT TTG CTG CAA TG 3'    | 5' TGC AGT TAA CTT GTT GAA AGG A 3' | CT    |
| WMS213 | 5' TGC CTG GCT CGT TCT ATC TC 3'    | 5' CTA GCT TAG CAC TGT CGC CC 3'    | GA    |
| WMS218 | 5' CGG CAA ACG GAT ATC GAC 3'       | 5' AAC AGT AAC TCT CGC CAT AGC C 3' | CT    |
| WMS219 | 5' GAT GAG CGA CAC CTA GCC TC 3'    | 5' GGG GTC CGA GTC CAC AAC 3'       | GAimp |
| WMS224 | 5' TGA GTC CAG CAC TGC TGC 3'       | 5' CAA CAT CCG CTC GTA TTC AA 3'    | CT    |
| WMS228 | 5' TCA TAT GCA CCT CTT TCC TAG G 3' | 5' GTG TGC CAC CTT TGA CGT C 3'     | CT,CA |
| WMS231 | 5' AGC TCG GGA TGA AGC GTG 3'       | 5' GAT CCG CCG CTG CGT TT 3'        | GAimp |

|        |                                      |                                      |            |
|--------|--------------------------------------|--------------------------------------|------------|
| WMS232 | 5' ATC TCA ACG GCA AGC CG 3'         | 5' CTG ATG CAA GCA ATC CAC C 3'      | GA         |
| WMS233 | 5' TCA AAA CAT AAA TGT TCA TTG GA 3' | 5' TCA ACC GTG TGT AAT TTT GTC C 3'  | CT         |
| WMS234 | 5' GAG TCC TGA TGT GAA GCT GTT G 3'  | 5' CTC ATT GGG GTG TGT ACG TG 3'     | CT,CA      |
| WMS237 | 5' GAA TCA CTT GTG AAG CAT CTG G 3'  | 5' CTG GAT GCA TCA CAT CCA AC 3'     | CT         |
| WMS238 | 5' TCG CTT CTA CCG CTC ACC 3'        | 5' AGT GCC TTG CCG AGG TC 3'         | CT,GT,GGGT |
| WMS241 | 5' TCT TCC AAC TAA AGC ATA GC 3'     | 5' CTT CCA TGG ACT ACA TAC TAG C 3'  | GA         |
| WMS242 | 5' TCC AAG GCA GTA GGC AGG 3'        | 5' TGT TGT TGG CCT GTA TGC AT 3'     | GA         |
| WMS244 | 5' GGC AGC TGA GGC AAT CTG 3'        | 5' TTT GGA CAT TTC CCA GCG 3'        | CAimp      |
| WMS245 | 5' CAG CGC AGT TAG CTC GC 3'         | 5' ATC TGT CCA TTC GAG CGC 3'        | CT         |
| WMS247 | 5' GCA ATC TTT TTT CTG ACC ACG 3'    | 5' ATG TGC ATG TCG GAC GC 3'         | GA         |
| WMS248 | 5' AGG ACT TCC GCA CCC TG 3'         | 5' TGG CGT GGT CTA AAT GGA C 3'      | CA         |
| WMS249 | 5' CAA ATG GAT CGA GAA AGG GA 3'     | 5' CTG CCA TTT TTC TGG ATC TAC C 3'  | GAimp      |
| WMS251 | 5' CAA CTG GTT GCT ACA CAA GCA 3'    | 5' GGG ATG TCT GTT CCA TCT TAG 3'    | CA         |
| WMS255 | 5' CAA CTG TAC GTA GGT TTC ATT GC 3' | 5' TCT GCC GTA AGT CGC CTC 3'        | GA         |
| WMS257 | 5' AGA GTG CAT GGT GGG ACG 3'        | 5' CCA AGA CGA TGC TGA AGT CA 3'     | GT         |
| WMS258 | 5' GAT CGC TTC ATC TCT CTC TCT C 3'  | 5' GTA CAC GCC GTA GGC CC 3'         | CT         |
| WMS259 | 5' AGG GAA AAG ACA TCT TTT TTT TC 3' | 5' CGA CCG ACT TCG GGT TC 3'         | GA         |
| WMS260 | 5' GCC CCC TTG CAC AAA TC 3'         | 5' CGC AGC TAC AGG AGG CC 3'         | GA         |
| WMS261 | 5' CTC CCT GTA CGC CTA AGG C 3'      | 5' CTC GCG CTA CTA GCC ATT G 3'      | CT         |
| WMS263 | 5' TCT GCC GTA AGT CGC CTC 3'        | 5' GGT TTC ATT GCT TGC CCT AA 3'     | CT         |
| WMS264 | 5' GAG AAA CAT GCC GAA CAA CA 3'     | 5' GCA TGC ATG AGA ATA GGA ACT G 3'  | CA         |
| WMS265 | 5' TGT TGC GGA TGG TCA CTA TT 3'     | 5' GAG TAC ACA TTT GGC CTC TGC 3'    | GT         |
| WMS268 | 5' AGG GGA TAT GTT GTC ACT CCA 3'    | 5' TTA TGT GAT TGC GTA CGT ACC C 3'  | GAimp      |
| WMS269 | 5' TGC ATA TAA ACA GTC ACA CAC CC 3' | 5' TTT GAG CTC CAA AGT GAG TTA GC 3' | CA         |
| WMS271 | 5' CAA GAT CGT GGA GCC AGC 3'        | 5' AGC TGC TAG CTT TTG GGA CA 3'     | CT,GA      |
| WMS272 | 5' TGC TCT TTG GCG AAT ATA TGG 3'    | 5' GTT CAA AAC AAA TTA AAA GGC CC 3' | CA         |
| WMS273 | 5' ATT GGA CGG ACA GAT GCT TT 3'     | 5' AGC AGT GAG GAA GGG GAT C 3'      | GA         |
| WMS274 | 5' AAC TTG CAA AAC TGT TCT GA 3'     | 5' TAT TTG AAG CGG TTT GAT TT 3'     | GT         |

|        |                                      |                                      |            |
|--------|--------------------------------------|--------------------------------------|------------|
| WMS275 | 5' AAT TTT CTT CCT CAC TTA TTC T 3'  | 5' AAC AAA AAA TTA GGG CC 3'         | CT         |
| WMS276 | 5' ATT TGC CTG AAG AAA ATA TT 3'     | 5' AAT TTC ACT GCA TAC ACA AG 3'     | CT         |
| WMS278 | 5' GTT GCT TCA TGA ACG CTC AA 3'     | 5' CTG CCC AAT TTT CTC CAC TC 3'     | GTimpGAimp |
| WMS281 | 5' CGG CCA TAT TTC TGT AAG TAT GC 3' | 5' GCA GGT AAT GGC CGG AC 3'         | GT         |
| WMS282 | 5' TTG GCC GTG TAA GGC AG 3'         | 5' TCT CAT TCA CAC ACA ACA CTA GC 3' | GA         |
| WMS284 | 5' AAT GAA AAA ACA CTT GCG TGG 3'    | 5' GCA CAT TTT TCA CTT TCG GG 3'     | GA         |
| WMS285 | 5' ATG ACC CTT CTG CCA AAC AC 3'     | 5' ATC GAC CGG GAT CTA GCC 3'        | GA         |
| WMS291 | 5' CAT CCC TAC GCC ACT CTG C 3'      | 5' AAT GGT ATC TAT TCC GAC CCG 3'    | CA         |
| WMS292 | 5' TCA CCG TGG TCA CCG AC 3'         | 5' CCA CCG AGC CGA TAA TGT AC 3'     | CT         |
| WMS293 | 5' TAC TGG TTC ACA TTG GTG CG 3'     | 5' TCG CCA TCA CTC GTT CAA G 3'      | CA         |
| WMS294 | 5' GGA TTG GAG TTA AGA GAG AAC CG 3' | 5' GCA GAG TGA TCA ATG CCA GA 3'     | GAimp      |
| WMS295 | 5' GTG AAG CAG ACC CAC AAC AC 3'     | 5' GAC GGC TGC GAC GTA GAG 3'        | GA         |
| WMS296 | 5' AAT TCA ACC TAC CAA TCT CTG 3'    | 5' GCC TAA TAA ACT GAA AAC GAG 3'    | CT         |
| WMS297 | 5' ATC GTC ACG TAT TTT GCA ATG 3'    | 5' TGC GTA AGT CTA GCA TTT TCT G 3'  | GT, GA     |
| WMS299 | 5' ACT ACT TAG GCC TCC CGC C 3'      | 5' TGA CCC ACT TGC AAT TCA TC 3'     | GA, TAG    |
| WMS301 | 5' GAG GAG TAA GAC ACA TGC CC 3'     | 5' GTG GCT GGA GAT TCA GGT TC 3'     | GA, G      |
| WMS302 | 5' GCA AGA AGC AAC AGC AGT AAC 3'    | 5' CAG ATG CTC TTC TCT GCT GG 3'     | GA         |
| WMS304 | 5' AGG AAA CAG AAA TAT CGC GG 3'     | 5' AGG ACT GTG GGG AAT GAA TG 3'     | CT         |
| WMS311 | 5' TCA CGT GGA AGA CGC TCC 3'        | 5' CTA CGT GCA CCA CCA TTT TG 3'     | GA         |
| WMS312 | 5' ATC GCA TGA TGC ACG TAG AG 3'     | 5' ACA TGC ATG CCT ACC TAA TGG 3'    | GA         |
| WMS313 | 5' CCG CCC TCA TTA AGT TTC AC 3'     | 5' TTT GAC AAG TAC ACG AGT CTG C 3'  | CT, GT     |
| WMS314 | 5' AGG AGC TCC TCT GTG CCA C 3'      | 5' TTC GGG ACT CTC TTC CCT G 3'      | CT         |
| WMS316 | 5' CAT GGA CAT TTT ACC ACA AGA C 3'  | 5' TGC GTG TGG TCC ACC TC 3'         | AT, GT     |
| WMS319 | 5' GGT TGC TGT ACA AGT GTT CAC G 3'  | 5' CCG GTG CTG TGT GTA ATG AC 3'     | CT         |
| WMS320 | 5' CGA GAT ACT ATG GAA GGT GAG G 3'  | 5' ATC TTT GCA AGG ATT GCC C 3'      | GT, GA     |
| WMS321 | 5' CAA TGT GGA GAC GGT GTG C 3'      | 5' TGT TGC ATG CGA TCA TGC 3'        | GT, GAimp  |
| WMS322 | 5' TCA CAA AAT GAT TTC TCA TCC G 3'  | 5' TGC AGA AAA CCA ACA AGG G 3'      | GA         |
| WMS325 | 5' TTT CTT CTG TCG TTC TCT TCC C 3'  | 5' TTT TTA CGC GTC AAC GAC G 3'      | CT         |

|        |                                     |                                      |              |
|--------|-------------------------------------|--------------------------------------|--------------|
| WMS328 | 5' GCA ATC CAC GAG AAG AGA GG 3'    | 5' CAC AAA CTC TTG ACA TGT GCG 3'    | GT           |
| WMS330 | 5' TTG CTA TCC ATG TGC CAG AG 3'    | 5' ACA TGT TTC ATG CAG GTA GCC 3'    | GTT          |
| WMS332 | 5' AGC CAG CAA GTC ACC AAA AC 3'    | 5' AGT GCT GGA AAG AGT AGT GAA GC 3' | GA           |
| WMS333 | 5' GCC CGG TCA TGT AAA ACG 3'       | 5' TTT CAG TTT GCG TTA AGC TTT G 3'  | GA           |
| WMS334 | 5' AAT TTC AAA AAG GAG AGA GA 3'    | 5' AAC ATG TGT TTT TAG CTA TC 3'     | GA           |
| WMS335 | 5' CGT ACT CCA CTC CAC ACG G 3'     | 5' CGG TCC AAG TGC TAC CTT TC 3'     | GA, GCGT     |
| WMS336 | 5' CCC TTT AAT CTC GCT CCC TC 3'    | 5' GTC TCT TTC TCG TAC TTC CAG G 3'  | CT           |
| WMS337 | 5' CCT CTT CCT CCC TCA CTT AGC 3'   | 5' TGC TAA CTG GCC TTT GCC 3'        | CT, CACT, CA |
| WMS339 | 5' AAT TTT CTT CCT CAC TTA TT 3'    | 5' AAA CGA ACA ACC ACT CAA TC 3'     | CT           |
| WMS340 | 5' GCA ATC TTT TTT CTG ACC ACG 3'   | 5' ACG AGG CAA GAA CAC ACA TG 3'     | GA           |
| WMS341 | 5' TTC AGT GGT AGC GGT CGA G 3'     | 5' CCG ACA TCT CAT GGA TCC AC 3'     | CT           |
| WMS342 | 5' TAT CCA GAG CAG ACG GAC G 3'     | 5' GGT CTA GCT TCG ACG ACA CC 3'     | GT           |
| WMS344 | 5' CAA GGA AAT AGG CGG TAA CT 3'    | 5' ATT TGA GTC TGA AGT TTG CA 3'     | GT           |
| WMS346 | 5' CAA GCA AGG TTT CGT TTT ATC C 3' | 5' GCA TGT GGT CCA TGT ACT GC 3'     | AT, GT       |
| WMS349 | 5' GGC TTC CAG AAA ACA ACA GG 3'    | 5' ATC GGT GCG TAC CAT CCT AC 3'     | GA           |
| WMS350 | 5' ACC TCA TCC ACA TGT TCT ACG 3'   | 5' GCA TGG ATA GGA CGC CC 3'         | GT           |
| WMS353 | 5' CCA TGT TGA GTA GGT TCA GCC 3'   | 5' CTT GGC CAG AAG CTA CGA AC 3'     | GCGT, GT     |
| WMS356 | 5' AGC GTT CTT GGG AAT TAG AGA 3'   | 5' CCA ATC AGC CTG CAA CAA C 3'      | GA           |
| WMS357 | 5' TAT GGT CAA AGT TGG ACC TCG 3'   | 5' AGG CTG CAG CTC TTC TTC AG 3'     | GA           |
| WMS358 | 5' AAA CAG CGG ATT TCA TCG AG 3'    | 5' TCC GCT GTT GTT CTG ATC TC 3'     | GAimp        |
| WMS359 | 5' CTA ATT GCA ACA GGT CAT GGG 3'   | 5' TAC TTG TGT TCT GGG ACA ATG G 3'  | CT, CTTimp   |
| WMS361 | 5' GTA ACT TGT TGC CAA AGG GG 3'    | 5' ACA AAG TGG CAA AAG GAG ACA 3'    | GAimp        |
| WMS368 | 5' CCA TTT CAC CTA ATG CCT GC 3'    | 5' AAT AAA ACC ATG AGC TCA CTT GC 3' | AT           |
| WMS369 | 5' CTG CAG GCC ATG ATG ATG 3'       | 5' ACC GTG GGT GTT GTG AGC 3'        | CTimp        |
| WMS371 | 5' GAC CAA GAT ATT CAA ACT GGC C 3' | 5' AGC TCA GCT TGC TTG GTA CC 3'     | CA, GA       |
| WMS372 | 5' AAT AGA GCC CTG GGA CTG GG 3'    | 5' GAA GGA CGA CAT TCC ACC TG 3'     | GA           |
| WMS374 | 5' ATA GTG TGT TGC ATG CTG TGT G 3' | 5' TCT AAT TAG CGT TGG CTG CC 3'     | GT           |
| WMS375 | 5' ATT GGC GAC TCT AGC ATA TAC G 3' | 5' GGG ATG TCT GTT CCA TCT TAG C 3'  | CA           |

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|        |                                      |                                     |            |
|--------|--------------------------------------|-------------------------------------|------------|
| WMS376 | 5' GGG CTA GAA AAC AGG AAG GC 3'     | 5' TCT CCC GGA GGG TAG GAG 3'       | CA, GAimp  |
| WMS382 | 5' GTC AGA TAA CGC CGT CCA AT 3'     | 5' CTA CGT GCA CCA CCA TTT TG 3'    | GA         |
| WMS383 | 5' ACG CCA GTT GAT CCG TAA AC 3'     | 5' GAC ATC AAT AAC CGT GGA TGG 3'   | GT         |
| WMS384 | 5' TTT TCA TTG TGC CCT CTA CT 3'     | 5' GCC AAG TTT CTT AGC TAG TTA A 3' | GTimp      |
| WMS388 | 5' CTA CAA TTC GAA GGA GAG GGG 3'    | 5' CAC CGC GTC AAC TAC TTA AGC 3'   | CT, CA, CA |
| WMS389 | 5' ATC ATG TCG ATC TCC TTG ACG 3'    | 5' TGC CAT GCA CAT TAG CAG AT 3'    | CT, GT     |
| WMS390 | 5' AAG TTT CAC ACA AGA TCT CTC C 3'  | 5' TGA CAA GTA CAC GAG TCT GC 3'    | CT, GT     |
| WMS391 | 5' ATA GCG AAG TCT CCC TAC TCC A 3'  | 5' ATG TGC ATG TCG GAC GC 3'        | CA, GA     |
| WMS393 | 5' TCA TCT GCT ATT TGT GCT ACA 3'    | 5' TCA AAT ACA CCA ATG TGC C 3'     | CA         |
| WMS395 | 5' TAC AAC CGC AAG TAA TGC CA 3'     | 5' TAC CAA CAC CCT AGC CCT TG 3'    | CA         |
| WMS397 | 5' TGT CAT GGA TTA TTT GGT CGG 3'    | 5' CTG CAC TCT CGG TAT ACC AGC 3'   | CT         |
| WMS400 | 5' GTG CTG CCA CCA CTT GC 3'         | 5' TGT AGG CAC TGC TTG GGA G 3'     | CA         |
| WMS403 | 5' CGA CAT TGG CTT CGG TG 3'         | 5' ATA AAT CAG TGC GGT CCA GG 3'    | CA         |
| WMS408 | 5' TCG ATT TAT TTG GGC CAC TG 3'     | 5' GTA TAA TTC GTT CAC AGC ACG C 3' | CA         |
| WMS410 | 5' GCT TGA GAC CGG CAC AGT 3'        | 5' CGA GAC CTT GAG GGT CTA GA 3'    | CA         |
| WMS411 | 5' CCC ATA CGA TGA TGT GTT TCC 3'    | 5' CAA ACG GAA CAT GGT CCC 3'       | CT         |
| WMS412 | 5' ATC AAC AAG GTT TGT GTG TTG G 3'  | 5' ATG AAA CGC GAC CTC CC 3'        | GA         |
| WMS413 | 5' TGC TTG TCT AGA TTG CTT GGG 3'    | 5' GAT CGT CTC GTC CTT GGC A 3'     | GA         |
| WMS415 | 5' GAT CTC CCA TGT CCG CC 3'         | 5' CGA CAG TCG TCA CTT GCC TA 3'    | GAimp      |
| WMS425 | 5' GAG CCC ACA AGC TGG CA 3'         | 5' TCG TTC TCC CAA GGC TTG 3'       | CT         |
| WMS427 | 5' AAA CTT AGA ACT GTA ATT TCA GA 3' | 5' AGT GTG TTC ATT TGA CAG TT 3'    | CA         |
| WMS428 | 5' CGA GGC AGC GAG GAT TT 3'         | 5' TTC TCC ACT AGC CCC GC 3'        | GA         |
| WMS429 | 5' TTG TAC ATT AAG TTC CCA TTA 3'    | 5' TTT AAG GAC CTA CAT GAC AC 3'    | CT         |
| WMS434 | 5' ATG AGT TCC GCC AAA GAA TG 3'     | 5' ACG AAA TAC ACA AGT GGG ACA 3'   | GT         |
| WMS437 | 5' GAT CAA GAC TTT TGT ATC TCT C 3'  | 5' GAT GTC CAA CAG TTA GCT TA 3'    | CT         |
| WMS440 | 5' CCT ATG GTC TCC ATC ATG AGG 3'    | 5' TCA TGT CAA CTC AAG AAC ACG 3'   | CT         |
| WMS443 | 5' GGG TCT TCA TCC GGA ACT CT 3'     | 5' CCA TGA TTT ATA AAT TCC ACC 3'   | CA, GA     |
| WMS445 | 5' TTT GTT GGG GGT TAG GAT TAG 3'    | 5' CCT TAA CAC TTG CTG GTA GTG A 3' | CT         |

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|        |                                     |                                     |              |
|--------|-------------------------------------|-------------------------------------|--------------|
| WMS448 | 5' AAA CCA TAT TGG GAG GAA AGG 3'   | 5' CAC ATG GCA TCA CAT TTG TG 3'    | GA           |
| WMS455 | 5' ATT CGG TTC GCT AGC TAC CA 3'    | 5' ACG GAG AGC AAC CTG CC 3'        | GTemp        |
| WMS456 | 5' TCT GAA CAT TAC ACA ACC CTG A 3' | 5' TGC TCT CTC TGA ACC TGA AGC 3'   | GA           |
| WMS458 | 5' AAT GGC AAT TGG AAG ACA TAG C 3' | 5' TTC GCA ATG TTG ATT TGG C 3'     | CA           |
| WMS459 | 5' ATG GAG TGG TCA CAC TTT GAA 3'   | 5' AGC TTC TCT GAC CAA CTT CTC G 3' | GA           |
| WMS469 | 5' CAA CTC AGT GCT CAC ACA ACG 3'   | 5' CGA TAA CCA CTC ATC CAC ACC 3'   | CT           |
| WMS471 | 5' CGG CCC TAT CAT GGC TG 3'        | 5' GCT TGC AAG TTC CAT TTT GC 3'    | CA           |
| WMS473 | 5' TCA TAC GGG TAT GGT TGG AC 3'    | 5' CAC CCC CTT GTT GGT CAC 3'       | GTemp        |
| WMS476 | 5' ATG GGT TCG TAC TAA CAT CAG C 3' | 5' TTG CTG GTA GCT TCA ATC CC 3'    | GTemp        |
| WMS480 | 5' TGC TGC TAC TTG TAC AGA GGA C 3' | 5' CCG AAT TGT CCG CCA TAG 3'       | CT, CA       |
| WMS484 | 5' ACA TCG CTC TTC ACA AAC CC 3'    | 5' AGT TCC GGT CAT GGC TAG G 3'     | CT           |
| WMS494 | 5' ATT GAA CAG GAA GAC ATC AGG G 3' | 5' TTC CTG GAG CTG TCT GGC 3'       | CA           |
| WMS495 | 5' GAG AGC CTC GCG AAA TAT AGG 3'   | 5' TGC TTC TGG TGT TCC TTC G 3'     | GA           |
| WMS497 | 5' GTA GTG AAG ACA AGG GCA TT 3'    | 5' CCG AAA GTT GGG TGA TAT AC 3'    | GTemp        |
| WMS499 | 5' ACT TGT ATG CTC CAT TGA TTG G 3' | 5' GGG GAG TGG AAA CTG CAT AA 3'    | GA           |
| WMS501 | 5' GGC TAT CTC TGG CGC TAA AA 3'    | 5' TCC ACA AAC AAG TAG CGC C 3'     | CA           |
| WMS512 | 5' AGC CAC CAT CAG CAA AAA TT 3'    | 5' GAA CAT GAG CAG TTT GGC AC 3'    | GT           |
| WMS513 | 5' ATC CGT AGC ACC TAC TGG TCA 3'   | 5' GGT CTG TTC ATG CCA CAT TG 3'    | CA           |
| WMS515 | 5' AAC ACA ATG GCA AAT GCA GA 3'    | 5' CCT TCC TAG TAA GTG TGC CTC A 3' | GTemp        |
| WMS518 | 5' AAT CAC AAC AAG GCG TGA CA 3'    | 5' CAG GGT GGT GCA TGC AT 3'        | CA           |
| WMS530 | 5' AAA TAG GAC AAC CCA CGG C 3'     | 5' TCA ACT TCT TGG CCT CCA TC 3'    | CT           |
| WMS532 | 5' ACT GCG TGT GCC TAC AAT TG 3'    | 5' TCA CTC GCA CTC GAT AGG C 3'     | GT           |
| WMS533 | 5' AAG GCG AAT CAA ACG GAA TA 3'    | 5' GTT GCT TTA GGG GAA AAG CC 3'    | CT, CA       |
| WMS537 | 5' ACA TAA TGC TTC CTG TGC ACC 3'   | 5' GCC ACT TTT GTG TCG TTC CT 3'    | CA, TA       |
| WMS538 | 5' GCA TTT CGG GTG AAC CC 3'        | 5' GTT GCA TGT ATA CGT TAA GCG G 3' | GTemp        |
| WMS540 | 5' TCT CGC TGT GAA ATC CTA TTT C 3' | 5' AGG CAT GGA TAG AGG GGC 3'       | CTTemp       |
| WMS544 | 5' TAG AAT TCT TTA TGG GGT CTG C 3' | 5' AGG ATT CCA ATC CTT CAA AAT T 3' | CT, ATCT, CT |
| WMS550 | 5' CCC ACA AGA ACC TTT GAA GA 3'    | 5' CAT TGT GTG TGC AAG GCA C 3'     | CT, GT       |



REGEL 26" SEITE 2630

WMS554  
WMS565  
WMS566  
WMS569  
WMS570  
WMS573  
WMS577  
WMS582  
WMS583  
WMS588

|                                     |                                     |           |
|-------------------------------------|-------------------------------------|-----------|
| 5' TGC CCA CAA CGG AAC TTG 3'       | 5' GCA ACC ACC AAG CAC AAA GT 3'    | CT, GTimp |
| 5' GCG TCA GAT ATG CCT ACC TAG G 3' | 5' AGT GAG TTA GCC CTG AGC CA 3'    | CA        |
| 5' TCT GTC TAC CCA TGG GAT TTG 3'   | 5' CTG GCT TCG AGG TAA GCA AC 3'    | CA, TA    |
| 5' GGA AAC TTA TTG ATT GAA AT 3'    | 5' TCA ATT TTG ACA GAA GAA TT 3'    | GT        |
| 5' TCG CCT TTT ACA GTC GGC 3'       | 5' ATG GGT AGC TGA GAG CCA AA 3'    | CT, GT    |
| 5' AAG AGA TAA CAT GCA AGA AA 3'    | 5' TTC AAA TAT GTG GGA ACT AC 3'    | CA        |
| 5' ATG GCA TAA TTT GGT GAA ATT G 3' | 5' TGT TTC AAG CCC AAC TTC TAT T 3' | CA, TA    |
| 5' AAG CAC TAC GAA AAT ATG AC 3'    | 5' TCT TAA GGG GTG TTA TCA TA 3'    | CA        |
| 5' TTC ACA CCC AAC CAA TAG CA 3'    | 5' TCT AGG CAG ACA CAT GCC TG 3'    | CA        |
| 5' GAT CCC CAA TTG CAT GTT G 3'     | 5' CTT GCA ACT GGG GGA CAC 3'       | GT        |

6. A method for the preparation of a microsatellite marker of claims 1 to 5 for plants of the *Triticum aestivum* species as well of the Tribe Triticeae, characterized in that hypervariable genome sections (so-called microsatellites), with the help of the polymerase chain reaction (PCR), are amplified, subsequently separated and detected to polymorphous fragments in the presence of two specific primers, which flank a microsatellite sequence to the left and right of each microsatellite locus.

7. The method of claim 6, characterized in that highly resolving agarose gels, native polyacrylamide gels or denaturing polyacrylamide gels are used for the separation of the markers.

8. The method of claim 6, characterized in that, depending on the separation system, the detection is carried out by means of ethidium bromide staining, silver staining, radiographic labeling followed by autoradiography or by means of automatic sequencing equipment using dye- or fluorescence-labeled primers.

9. The use of the microsatellite markers of claims 1 to 7, for the genetic analysis of hexaploid and tetraploid cultivated forms of wheat.

10. The use according to claim 8 for the genetic mapping and marking of monogenic and polygenic properties and their selection for analyzing relationships and identifying varieties, as well as for evaluating the purity of varieties, identifying hybrids and breeding plants.

Applicant or Patentee: \_\_\_\_\_  
Serial or Patent Number: \_\_\_\_\_ Attorney's Docket No. \_\_\_\_\_  
Filed or Issued: \_\_\_\_\_  
For: \_\_\_\_\_

Verified Statement (Declaration) Claiming SMALL ENTITY  
Status (37 CFR 1.9(f) and 1.27 (d)) - Small Business Concern

PHOSPHORUS PATENT 1149

I hereby declare that I am

☒ the owner of the small business concern identified below  
☐ an official of the small business concern empowered to act on behalf of the concern identified below

NAME OF CONCERN Institut für Pflanzengenetik und Kulturpflanzenforschung  
ADDRESS OF CONCERN Corrensstrasse 3, D-06466 Gatersleben, Germany

I hereby declare that the above identified small business concern qualifies as a small business concern as defined in 13 CFR 121.3-18, and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention, entitled Microsatellite Markers for Plants of the Species Triticum Aestivum and Tribe Triticeae and the Use of Said Markers by inventor(s) Marion Röder, Jens Plaschke, and Martin Ganal described in

☐ The specification filed herewith  
☐ application serial no. \_\_\_\_\_, filed \_\_\_\_\_  
☐ patent no. \_\_\_\_\_, issued \_\_\_\_\_

If the rights held by the above identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed below\* and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

\*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

NAME: \_\_\_\_\_  
ADDRESS: \_\_\_\_\_  
☐ Individual ☐ Small Business Concern ☐ Nonprofit Organization

NAME: \_\_\_\_\_  
ADDRESS: \_\_\_\_\_  
☐ Individual ☐ Small Business Concern ☐ Nonprofit Organization

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING: Prof. Dr. Ulrich Wobus Bernd Eise  
TITLE OF PERSON OTHER THAN OWNER: Acting Director Admin. Director  
ADDRESS OF PERSON SIGNING: Institute of Plant Genetics and Crop  
Plant Research, Corrensstrasse 3, 06466 Gatersleben, Germany

SIGNATURE [Signature] DATE 10. Jan. 1998

**LONDA AND TRAUB LLP**  
Wall Street Tower  
20 Exchange Place - 37th Floor  
New York, New York 10005  
United States of America

If each inventor understands English, the Declaration and Power of The Attorney below is suitable for use when filing regular patent application and also when entering the national stage, in the case of an International application designating the USA under the PCT.

CAVEAT: Please read accompanying INFORMATION SHEET before signing

**COMBINED DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION**

Attorney Docket No.  
2935.10400

As a below named inventor, I hereby declare that:  
My residence, post office address and citizenship are as stated below next to my name,  
I believe I am the original, first and sole inventor (if only one name is listed below at 201) or an original, first and joint inventor (if plural names are listed below at 201-205) of the subject matter which is claimed and for which a patent is sought on the invention entitled

Microsatellite Markers for Plants of the Species Triticum Aestivum and Tribe Triticeae and the Use of Said Markers

the specification of which (check one)

☐

is attached hereto

☒

was filed on 27 June 1996

under Serial Number PCT/DE96/01185

and was amended on (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, Section 1.56

I list below any prior foreign application(s) for patent or inventor's certificate in respect of which foreign priority benefits are claimed under 35 USC 119; and any prior foreign application(s) for patent or inventor's certificate in respect of which such foreign priority rights are not claimed and which has a filing date before that of any application in respect of which such foreign priority benefits are claimed:

| Application Number | Country | Filing Date<br>(day,month,year) | Priority Claimed under<br>35 USC 119 |
|--------------------|---------|---------------------------------|--------------------------------------|
| 195 25 284.5       | Germany | 28.6.95                         | YES: <u>X</u> NO: <u>  </u>          |
|                    |         |                                 | YES: <u>  </u> NO: <u>  </u>         |

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below.

Application No.

Filing Date

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

Bruce S. Londa (33,531) Brian L. Wamsley (33,045)  
Alex L. Yip (34,759)

|            |   |  |  |
|------------|---|--|--|
| <b>201</b> | Family Name<br><u>RODER</u>                   | First Given Name<br><u>Marlon</u>                        | Second Given Name                        |
|            | City of Residence<br><u>Rieder</u>            | State or Foreign<br>Country<br><u>Germany</u> <u>DEX</u> | Country of Citizenship<br><u>Germany</u> |
|            | Post Office Address<br><u>Reuthestrasse 9</u> | City<br><u>D-06507 Rieder</u>                            | State & ZIP/Country<br><u>Germany</u>    |
|            |   |  |  |

Continued

|                            |   |   |  |
|----------------------------|---|---|--|
| <b>202</b> <sup>2-00</sup> | Family Name<br><u>PLASCHKE</u>                  | First Given Name<br><u>Jens</u>                           | Second Given Name                        |
|                            | City of Residence<br><u>Meissen</u>             | State or Foreign Country<br><u>Germany</u> <sup>DEX</sup> | Country of Citizenship<br><u>Germany</u> |
|                            | Post Office Address<br><u>A.-Mücke-Ring 12B</u> | City<br><u>D-01662 Meissen</u>                            | State & Zip/Country<br><u>Germany</u>    |
| <b>203</b> <sup>3-00</sup> | Family Name<br><u>GANAL</u>                     | First Given Name<br><u>Martin</u>                         | Second Given Name                        |
|                            | City of Residence<br><u>Rieder</u>              | State or Foreign Country<br><u>Germany</u> <sup>DEX</sup> | Country of Citizenship<br><u>Germany</u> |
|                            | Post Office Address<br><u>Reuthestrasse 9</u>   | City<br><u>D-06507 Rieder</u>                             | State & Zip/Country<br><u>Germany</u>    |
| <b>204</b>                 | Family Name                                     | First Given Name  | Second Given Name                        |
|                            | City of Residence                               | State or Foreign Country                                  | Country of Citizenship                   |
|                            | Post Office Address                             | City  | State & Zip/Country                      |
| <b>205</b>                 | Family Name                                     | First Given Name  | Second Given Name                        |
|                            | City of Residence                               | State or Foreign Country                                  | Country of Citizenship                   |
|                            | Post Office Address                             | City  | State & ZIP/ Country                     |

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

|                           |                      |      |                       |
|---------------------------|----------------------|------|-----------------------|
| Signature of Inventor 201 | <u>Marion Riedel</u> | Date | <u>Jan. 7th, 1998</u> |
| Signature of Inventor 202 | <u>Jens Planchke</u> | Date | <u>Jan. 12, 1998</u>  |
| Signature of Inventor 203 | <u>Martin Ganal</u>  | Date | <u>Jan. 7th, 1998</u> |
| Signature of Inventor 204 |                      | Date |                       |
| Signature of Inventor 205 |                      | Date |                       |

105 Rec'd PCT/PTO 01 MAY 1998

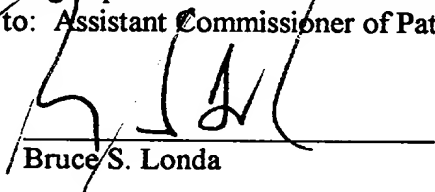
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PATENTS

MAILING CERTIFICATION

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Assistant Commissioner of Patents, Washington, D.C. 20231 on April 29, 1998

  
Bruce S. Londa

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Atty's Docket No. 2936.104/00

Applicant : Marion Roder

Appln. Number : 08/983,605

Filed : 12/29/97

For: Microsatellite Markers for Plants of the Species *Triticum Aestivum* and Tribe Triticeae and the Use of Said Markers

BOX PCT

Hon. Assistant Commissioner of Patents  
Washington, D.C. 20231

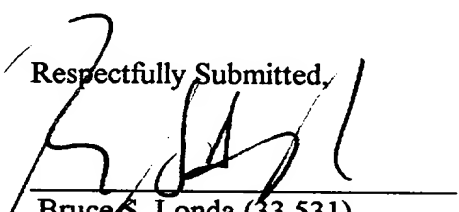
Sir:

Applicant submits herewith the Declaration required under 37 CFR 1.63.

Kindly charge the surcharge of \$65.00, applicant(s) being entitled to Small Entity Status on the basis of Verified Statement(s) filed February 13, 1998, to Account No. 04-2216.

The Commissioner is hereby authorized to charge any additional fees which may be required to make this response timely, or credit any overpayment to Deposit Account 04-2216.

Respectfully Submitted,

  
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